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Edited at The University of Texas Medical Branch, Galveston

THE BILIARY EXCRETION OF GALLIUM^{72}, GOLD^{198}, AND HAFNIUM^{181} IN THE RAT*

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Introduction

The following study was made to estimate the effects of relatively small quantities of radioactive materials in tracer amounts on early bile production by the liver, as well as the excretion of these materials in the bile at short intervals after their introduction into the animal. These experiments offered an opportunity to measure any immediate impairment of liver function as measured by bile production, in addition to the ability of the liver to retain or eliminate the respective materials. Radioactive colloidal gold^{198}, gallum^{72} citrate, hafnium^{181} sodium mandelate, and hafnium^{181} catechol disulfonate were used. The hafnium compounds were prepared by a method furnished by Dr. Carl T. Bahner, Department of Chemistry, Carson-Newman College, Jefferson City, Tennessee.

Procedure

The experimental animal was the adult Wistar rat; both sexes being used. Bile was collected from the animal in the anesthetized state. A combination of sodium pentobarbital and sodium phenobarbital was used as the anesthetic agent. The bile duct was cannulated as follows: A small abdominal incision was made in the epigastric region, the duodenum with the hepatic duct leading to it was located, and an opening made in the duodenum. Polyethylene tubing of adequate size was introduced into the hepatic duct and one end of the tubing was exteriorized.

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The incision was clamped, and the bile collected in a conical centrifuge tube.

The radioactive material was injected by tail vein immediately after bile duct cannulation; usually the volume administered was 0.2 ml. When gallium citrate was injected, 0.5 ml. of 10% calcium gluconate or 5% calcium chloride was added to replace the calcium ion which had become immobilized by the citrate ion. The volume of bile secreted was measured during a given time interval; this volume was brought up to 10 ml. for counting by adding water. The sample was placed in a small Petri dish and the activity measured. For the colloidal gold^{198} and gallium^{72} citrate a lead-walled, horizontal counting chamber with a thin glass-walled Geiger-Mueller tube was employed. For the hafnium^{181} compounds a vertical counting chamber with a thin mica window Geiger-Mueller tube was used. Preceding removal of the liver, the animal was perfused with isotonic saline via the renal artery and the inferior vena cava. The liver was weighed, digested with concentrated nitric acid, diluted to a known volume, and a 10 ml. aliquot counted.

A series of experiments were conducted to estimate the liver and blood concentrations of the gallium^{72} citrate at varying intervals after injection and to ascertain if these followed the biliary excretion pattern. In these experiments the animals were anesthetized as previously described and 1 ml. of blood was drawn from the inferior vena cava, diluted to 10 ml., and counted. Liver activity was estimated as described above.

Data obtained in these experiments are presented in accompanying tables. The activities of all compounds, except those of hafnium, are expressed as counts per minute after correction to zero time. The latter is not identical in all cases.

Results and Discussion

Colloidal Gold^{198} Experiments: Five rats were used; the data from three of these are presented in Table I. The liver activity was measured in these animals. It is evident that liver activity was high although no appreciable amount of radioactive gold appeared in the bile. The ability of the reticulo-endothelial system of the liver to retain colloidal gold at least for the first two hours

TABLE I

Colloidal Gold^{198}

Animal	Wt.		Bile 1st 60 min.	Bile 2nd 60 min.	Liver counts/min./gm.
No. 3		vol. bile (ml.)	.60	.40	
♀	177 gms.	counts/min.	23	26	22,857
No. 4		vol. bile (ml.)	.55	.48	
♀	165 gms.	counts/min.	24	24	27,764
No. 5		vol. bile (ml.)	.50	.30	
♀	170 gms.	counts/min.	24	24	32,073

is apparent. It may be observed that in all three animals there was a decreased secretion of bile in the second hour, however, (Table 5) this was generally the case in all animals studied.

Gallium^{72} *Citrate Experiments:* Five rats were used; the data from three animals are presented in Table II. It is evident that

TABLE II

Gallium^{72} *Citrate*

Animal	Wt.		Minutes 15	30	45	60	75	90	105	120	Liver counts /min./gm.
No. 3		vol. bile (ml.)	.15	.18	.18	.12	.26	.30	.23	.19	
♂	197 gms.	counts/min.	882	400	312	291	483	443	191	168	3,354
No. 4		vol. bile (ml.)	.17	.17	.16	.13	.23	.23	.23	.21	
♂	170 gms.	counts/min.	2,195	527	445	345	512	377	329	259	4,404
No. 5		vol. bile (ml.)	..	.37*	..	.43*	.38	.34	.30	.28	
♀	250 gms.	counts/min.	..	7,067*	..	1,476*	2,163	2,393	1,656	1,164	18,072

*Represents 30 minute intervals.

a different situation exists from the colloidal gold^{198} experiments. An appreciable amount of gallium activity appeared in the bile; the greatest amount being present in the first fifteen minutes of secretion, and there was generally a progressive decrease in activity, except after the first 60–90 minutes there was a slight increase in activity, then a decline.

Blood and Liver Levels: In order to compare the blood and liver levels with the biliary excretion pattern, eleven rats were used, the data of which are presented in Table III. In this experiment each animal was injected with 0.2 ml. of gallium^{72} citrate; the blood, and the liver were measured for activity at varying intervals after injection.

As seen in Table III there is a progressive decrease in gallium^{72} activity in both blood and liver, and the decrease in

TABLE III

Gallium^{72} *Citrate**

No. Rats Used	Time (Min.)	Blood counts/min./ml.†	Liver counts/min./gm.
2	8	8.07×10^4	1.99×10^4
2	15	5.87×10^4	1.51×10^4
2	30	3.40×10^4	1.08×10^4
2	60	1.38×10^4	8,370
1	120	1.72×10^4	7,780
2	180	9,920	7,490

*Values in all cases except one are averages of two animals.
†Identical zero time used in all counts for blood and liver.

activity occurs concurrently with the drop of bile activity. The liver did not retain the gallium^{72} citrate or a converted compound of gallium,^{72} as in the case of colloidal gold.[198] It appears that the concentration of activity of gallium^{72} in the bile and liver follows the concentration of activity in the blood.

Hafnium Experiments: Two rats were injected with hafnium^{181} sodium mandelate an done was injected with hafnium^{181} catechol disulfonate. Data are presented in Table IV. Here again

TABLE IV

Hafnium^{181} *Sodium Mandelate*

Animal	Wt.		Bile 15 Min.	30 Min.	60 Min.	120 Min.	Liver counts/min./gm.
No. 1		vol. bile (ml.)	.11	.13	.20	.36	
♀	186 gms.	counts/min.	8	23	39	31	7.3×10^4
No. 2		vol. bile (ml.)	.14	.13	.19	.36	
♀	176 gms.	counts/min.	33	25	70	104	4.99×10^4

Hafnium^{181} *Catechol Disulfonate*

Animal	Wt.		Bile 15 Min.	30 Min.	60 Min.	120 Min.	Liver counts/min./gm.
No. 3		vol. bile (ml.)	.10	.19	.27	.45	
♀	180 gms.	counts/min.	28	84	101	90	1.53×10^4

a manifestation of the reticulo-endothelial system of the liver is noted. Although the liver concentration of the hafnium^{181} compounds was high, the bile count is only slightly higher than background activity.

Control Experiments: In Table V the volume secretion of bile is shown. Apparently the radioactive tracer compounds in the quantities used did not appreciably change the volume of bile secreted.

TABLE V

Bile Secreted

Animals	Microcuries Injected	(ml. per 100 grams body weight*) 1st Hr.	2nd Hr.	3rd Hr.
Control (6♀)	....	.32	.25	.26
Colloidal Gold^{198}				
(3♀)	17 to 27	.32	.23	..
(2♂)	9 to 12	.35	.33	..
Gallium^{72} Citrate				
(1♀)	480	.32	.29	.23
(4♂)	10 to 125	.36	.27	.24†
Hafnium^{181} Sodium Mandelate				
(2♀)	33	.25	.20	..
Hafnium^{181} Catechol Disulfonate				
(1♀)	35	.31	.25	..

*Values are average except in one case.
†Average of two animals.

Summary

Under the conditions of these experiments:

(1) Practically no colloidal gold^{198} was excreted in bile although liver activity was comparatively high. This would indicate that colloidal gold^{198} is held by the reticulo-endothelial system of the liver.

(2) Gallium^{72} citrate or a gallium^{72} compound was excreted in bile. Evidently it is not held by the liver, and the concentrations in bile and liver closely follow the decrease of activity in the blood.

(3) The hafnium^{181} compounds used in these experiments are evidently retained by the liver similarly to the colloidal gold ^{198}.

(4) Liver function, as measured by volume of bile secreted, did not appear to be impaired for the short periods of time as described in these experiments.

A METHOD FOR RADIOAUTOGRAPHIC LOCALIZATION OF ISOTOPES* IN TISSUES†

H. W. Diserens and O. Hall

An investigation on the permeability of frog skin to phosphate ion (as radiophosphorus) revealed that skin exposed to this tracer incorporated a quantity of isotope which could not be removed by thorough washing in frog Ringer's solution (Diserens, 1951). Presumably, the phosphate was in a bound state. From results obtained in these experiments which point out the marked impermeability of the skin to this ion, it became increasingly important to attempt localization of the isotope. Radioautography was indicated as the only feasible method.

Radioautographic methods offer an excellent histochemical approach as an adjunct to ion balance or permeability studies. Many of the early methods employed a technic whereby a section of tissue or a gross structure was placed on an emulsion resulting in the production of an autograph (Leblond, 1943; Hamilton, 1942; Leblond, 1943a). Removal of tissue from the emulsion allowed visualization of autograph and tissue as mirror images. Such methods lend themselves as excellent means for the localization of general areas but fail to suffice for cellular detail due primarily to a failure to gain sufficient contiguity of tissue and emulsion. Leblond *et al.* (1948) describe a superior method in which the emulsion from medium lantern slides was temporarily liquefied and applied over a section of tissue containing tracer. Better resolution was obtained and also the simultaneous visualization of tissue and autograph was facilitated. However, difficulty in this instance would appear to stem primarily from variations in emulsion thickness and the inclusion of air bubbles, which factors obscure detail. Prestaining procedures in many methods heretofore used result in so great a loss of the isotope as to demand extremely high initial activity in order to expose the emulsion. Excellent reviews of the methods

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†From the Carter Physiology Laboratory, The University of Texas Medical Branch, Galveston. Received for publication February 23, 1952.

of radioautography are those given by Yagoda (1949) and more recently by Leblond and Gross (in Hahn, 1951).

Summarily, any autographic method meets with two impositions: (1) methods of fixation and staining must not result in so great a loss of the isotope as to make it virtually impossible to gain sufficient exposure, and (2) the tissue must bear staining for cellular detail. Staining after development therefore is preferable. The method herein described appears to satisfy these two essential requirements without obvious sacrifice to either. Production of autographs using two different isotopes and two different tissues has been accomplished by the use of identical procedures.

Methods and Materials. 1. Emulsion. Type NTB_2 emulsion as Nuclear Track Plates developed in the Kodak Laboratories was selected for use in the present study. Emulsions available from this and other companies can be obtained in a broad spectrum of characteristics to satisfy a variety of conditions dependent on the isotope used. Plates used had an emulsion thickness of 10μ surfaced on a 1″ x 3″ microscope slide and were obtained without a gelatin covering. Though the gelatin covering should theoretically and presumably practically aid in avoiding abrasion fog, it is felt that careful handling eliminates this difficulty and its absence permits closer contiguity of tissue and emulsion. Hence, an emulsion of uniform thickness, without air bubbles, mounted on a microscope slide promotes ease of handling and appears to circumvent many of the limitations of earlier methods.

2. Tracer technic. *Frog Skin.* Abdominal skin from the frog *Rana pipiens* was removed from pithed animals and mounted between two pyrex glass chambers which had been made with air inlet tubes, sampling arms, electrode outlet tubes and with a ground-glass taper joint. Skin placed between these chambers completely separated the solutions on either side therefore preventing exchange of water or dissolved materials except through the skin. Skin thus arranged was subjected to 30 microcuries of radiophosphorus (as NaH_2PO_4) in frog Ringer's solution placed on the inside of the skin for a period of 2 hours. Frog Ringer's was in contact with the outside of the skin surface. On termination of the experiment, skin was removed from the chamber, the small portion exposed to tracer isolated and thoroughly washed

in Ringer's, pH 7.0, until washings contained no radioactive ion. The skin was then ready for fixation.

Rat thyroid. A female, albino rat weighing 198 grams was injected intravenously through a tail vein with 73 microcuries of I^{131} in normal blood serum having the globulin fraction labeled with the isotope. The animal was sacrificed three hours later and the thyroid removed. One half of the thyroid was taken for fixation whereas the remaining half was dissolved in concentrated HNO_3 and the level of isotope present determined by means of the Geiger-Müller counter so that an idea of the amount of activity contained in the tissue could be gained.

3. Fixation. Frozen tissue sections afford a method whereby loss of isotope is negligible (Holt and Warren, 1950) however, they present a limitation when applied to autographic technics since resolution obtained is inferior to methods employing fixation followed by paraffin embedding and sectioning. In the case of soluble P^{32} a small amount of isotope is lost when tissues are fixed in neutral formol though the loss appears negligible if the period of fixation is held to a minimum and if the solution is maintained at a neutral pH. Neutral formol (10%) was therefore chosen as fixative. Immediately after washing in Ringer's, skin was pinned on to a small piece of cork to prevent curling and placed in fixative. Thyroid was placed in fixative immediately after excision.

Complete procedures for fixing, dehydrating and sectioning of the tissues are given in Table I. Frog skin and rat thyroid were treated in an identical manner.

4. Application of sections to emulsion. Approximately 12 sections were made for each plate to be exposed. A few additional sections were prepared for regular staining procedures and mounted on ordinary microscope slides. These served for comparison. Once cut, sections were floated onto the surface of about 100 ml. of distilled water at 42° C. contained in a 125 ml. beaker. Sections were floated in as many beakers as the number of plates to be used. These were removed to the darkroom for application of sections to plates. Total dark is preferable for work in the case of an emulsion of sensitivity such as used here. A Wratten Series No. 2 filter could be used but at a minimum distance of 4 feet

TABLE I

Fixation, dehydration, and sectioning procedures for tissues containing isotope preparatory to mounting on emulsion

Procedure	Time (in min.)
Fix, Neutral Formol (10%)	120
Wash (distilled water), 3 changes	2
Dehydrate (alcohols)	
70% ethyl	10
90% ethyl	10
95% ethyl	10
99% isopropyl	15
Absolute ethyl	5
Xylol	10
Infiltrate with paraffin	30
Embed, block, and section*	

*Sections 8, 10, 12 and 14 μ thick were cut using a rotary microtome.

which affords little light at the working area. Total darkness has imposed no severe handicap in the handling of the slides when application of sections to slides was made in the following manner. Operations in the darkroom were best carried out by two people. After the beakers had been arranged in a predetermined position on a table the lights were turned out. One person then handed a single slide, emulsion side up, to a second person who immediately slid the plate vertically downward along the side of the beaker and withdrew it at an angle of about 45°, thus drawing the sections on to the emulsion. The lower edge of the slide was placed on filter paper at a tilt and allowed to air dry. When dry, slides were stored in a light-tight box for the exposure period in a room held at approximately 70° F. and at a relative humidity of about 50%. No drying agent was used in the storage box.

5. Exposure period. Great difficulty is encountered in calculating the length of time required for suitable exposure of the emulsion to radiant energy of the isotopes, as this is influenced by the activity of the tissue and the sensitivity of the emulsion. Isotopes are in general bound in specific areas within a tissue thus making the problem more complex. In this study exposure times were roughly calculated from the values of the activity obtained

within a given amount of ashed tissue. Such a method has inherent many errors since the assumption that the isotope is uniformly distributed throughout the tissue is invalid. An empirical derivation appears preferable. Several autographs were made and developed at various times. Exposure period for the autograph of frog skin shown in Fig. 1 was 12 days, whereas that of the rat thyroid was 5 days.

6. Developing procedures. At a time either calculated for sufficient exposure or arbitrarily selected, the autographs were developed according to the procedures outlined in Table II.

TABLE II

Procedures for developing Nuclear Track Plates (NTB$_2$, 10μ, Kodak) exposed to radioactive isotopes P^{32} and I^{131} contained in frog skin and rat thyroid, respectively

Procedure	Time (in min.)	Temp. °F.
Type Developer		
Kodak D 19 (concentrated)	4	68*
Stop bath, running tap water (gentle)	10	68
Acid Fixer, Stage 1	60†	68
Paraffin removal		
70% ethyl alcohol	1	
99% isopropyl alcohol	1	
Xylol	1	
99% isopropyl alcohol	1	
70% ethyl alcohol	1	
Acid Fixer, Stage 2	30‡	68
Wash, running tap water	60	..
Air dry, flat	..	..

Note: All procedures to the fixing stage carried out in total darkness. Light from Wratten Series No. 2 used to ascertain completeness of clearing after the initial fixing stage.

*As recommended by Kodak.
†View for completeness of clearing using Wratten No. 2. If clear with exception of area under sections, remove paraffin, if not, return to fixer.
‡Or until emulsion is clear all over.

Temperatures of the solutions used in the process of development of the exposed emulsion do not appear critical since in certain cases these exceeded the 68° F. temperature recommended

by about 5-8 degrees. Neither peeling nor cracking of the emulsion resulted from such variations. However, the maximum permissible variation has not been determined.

7. Staining of sections on the developed plate. One marked disadvantage occurs with the use of hematoxylin and eosin staining procedures, namely the emulsion stains so intensely as to obscure details in the section. Use of *in toto* stains has been advocated by Evans (1947), however, no plates were shown and the procedures for the process were not given. In the present study alum cochineal, which stained the tissue without excessively staining the emulsion, was used. It has proved of further convenience in that no counter-stain was required. It was found that alum cochineal flooded onto the surface of the sections on the emulsion resulted in a stained preparation in 15 minutes. Procedures for staining the sections on the developed emulsion are given in Table III.

TABLE III

Staining and dehydration procedures for sections on developed Nuclear Track Plates

Procedure	Time (in min.)
Flood plate with alum cochineal	15
Wash, distilled water	5
Dehydration (alcohols)	
50% ethyl	2
70% ethyl	2
95% ethyl	2
99% isopropyl	2
Xylol, 2 changes	2
Mount in gum damar	

Discussion. Resolution obtained would appear to be greatly dependent on the juxtaposition of the tissue and emulsion. In the method outlined close contiguity is achieved by a direct application of sections to the emulsion. Photomicroautographs of both frog skin and rat thyroid are shown in Figs. 1 and 2, respectively.

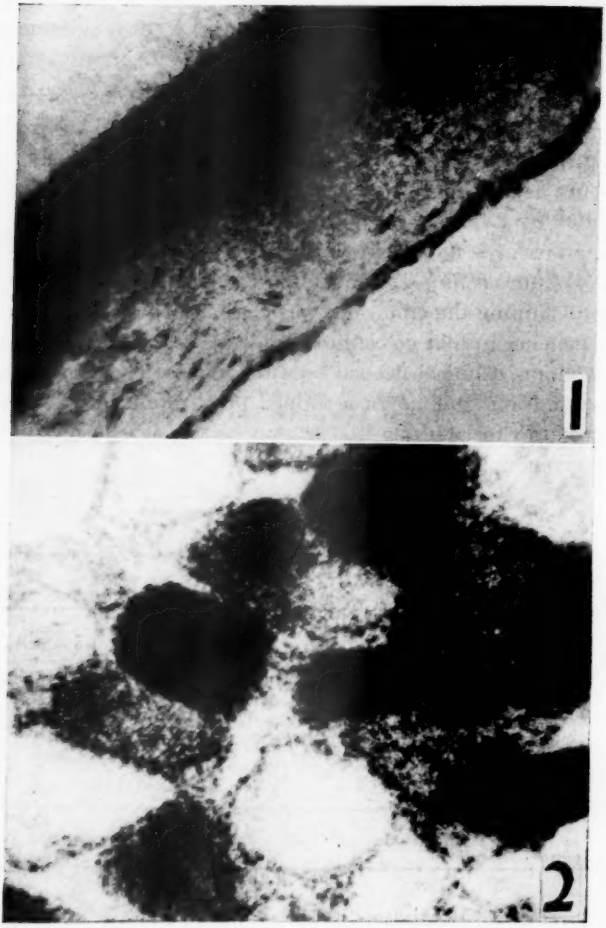

FIG. 1. Radioautograph of abdominal skin of frog, *Rana pipiens*. Isolated skin was exposed to 30 microcuries of P^{32} for 2 hours. Tissue was fixed in neutral formol and stained with alum cochineal. NTB_2 Nuclear Track Plates (Kodak) were exposed 12 days. Magnification 250X.

FIG. 2. Radioautograph of rat thyroid. Animal sacrificed 3 hours after intravenous injection of 73 microcuries of I^{131}. Fixing and staining procedure as in Fig. 1. Plates exposed 5 days. Magnification 250X.

Some stray radiation is seen to exist in the frog skin, Fig. 1, far more apparently than in the thyroid gland. This scattering of radiation may have resulted from a slight period of overexposure imposed by the relatively low content of activity in the skin. Concentration of the isotope in the region just below the basement membrane of the skin far exceeds that in other layers and it is presumed that binding occurred primarily to tissues in this area.

In the case of the autograph of rat thyroid the concentration of I^{131} is undeniably in the colloid. However, the concentration of the iodine within various colloid spaces varies and hence the amount and nature of colloid in the spaces would appear to differ.

Major advantages to be derived from the present technic are: 1. Production of the autograph and staining procedures are independent of each other. 2. Tissue and autograph may be viewed simultaneously. 3. Staining procedures employed require a minimum of time and the emulsion does not stain so intensely as to obscure detail of cellular components. 4. Use of Nuclear Track Plates (Kodak) permits a close juxtaposition of tissue and emulsion throughout the entire procedures by direct application of sections to the emulsion. 5. This simplified method is rapid and precise and results in autographs with superior resolution when compared to those produced by some of the earlier methods.

Conclusions. 1. A method for the production of radioautographs equally suitable for two different types of tissue and two isotopes is described in detail. 2. NTB_2 emulsion (Kodak) as nuclear track plates with a 10μ thickness without gelatin covering was used. Sections of tissues were made after exposure to the isotopes and applied to the emulsion. Development of the image produced by the isotopes was followed by staining of the sections using alum cochineal. No counter-stain was required.

REFERENCES

Diserens, H. W.: Relation of EMF to permeability of frog skin to phosphorus. *Fed. Proc.*, 10, 36, 1951.

Evans, T. C.: Radioautographs in which the tissue is mounted directly on the photographic plate. *Proc. Soc. Exp. Biol. & Med.*, 64, 313–315, 1947.

Hahn, P. F.: *A Manual of Artificial Radioisotope Therapy*, Academic Press, Inc., New York, 250–273, 1951.

Hamilton, J. G.: The use of radioactive tracers in biology and medicine. *Radiology*, 39, 541–572, 1942.

Holt, M. W., and Warren, S.: The radioautographic method for detailed localization of radioactive isotopes in tissues without isotope loss. *Proc. Soc. Exp. Biol. & Med.*, 73, 545–549, 1950.

Leblond, C. P.: Locating iodine in tissues autographically, especially after fixation by freezing and drying. *Stain Technology*, 18, 159–164, 1943.

Leblond, C. P.: Localization of newly administered iodine in the thyroid gland as indicated by radio-iodine. *J. Anatomy*, 77, 149–152, 1943a.

Leblond, C. P., Percival, W. L., and Gross, J.: Autographic localization of radio-iodine in stained sections of thyroid gland by coating with photographic emulsion. *Proc. Soc. Exp. Biol. & Med.*, 67, 74–76, 1948.

Yagoda, H.: *Radioactive Measurements with Nuclear Emulsions*. John Wiley and Sons, Inc., New York, 1949.

THE NEUROTOXIN OF *SHIGELLA DYSENTERIÆ*: STATEMENT OF PROBLEM AND REVIEW OF THE LITERATURE

FRANK B. ENGLEY, JR.*

The toxic properties of *Shigella dysenteriae* have been the subject of considerable controversy for nearly half a century. In particular, the neurotoxic factor produced by the organism has been the cause of dissension and discussion, as a study of textbooks by Topley and Wilson (1946), Felsen (1945) and Dubos (1945), and special articles by Weil (1943, 1947a, 1947b) and Pillemer (1949) reveals. This paper has as its purpose to review, from the discovery of the organism, the work on *Shigella dysenteriae* relative to the neurotoxic factor.

Although Chantemesse and Widal (1888) and Grigorjeff (1892) probably isolated the causative agent of bacillary dysentery before Shiga (1898), it was Shiga who, nevertheless, in his classical report on the etiology of the disease with the isolation of a gram negative bacillary organism from a Japanese epidemic in 1896, gave the conclusive evidence. As a result, the genus *Shigella* stems from his name. Soon after Shiga's discovery, Kruse (1900) in Germany, Flexner (1900) in the Philippines and a host of others, in the next few years, isolated the organism and other similar species in various parts of the world. This genus contains numerous species, one of which *Shigella paradysenteriae* Flexner, has many types, as shown by serological methods (Weil, 1947).

Conradi (1903), about five years following the discovery of the organism by Shiga, reported that autolysates of 18 hour agar grown cultures of *Shigella dysenteriae* would kill animals. His method consisted of suspending the cells in saline at 37° C. for 24–48 hours, diluting five-fold, filtering and concentrating at

*This paper is based on work done at the Biological Laboratories, Chemical Corps, Camp Detrick, Frederick, Maryland and presented in partial fulfillment of the requirements for the Degree of Doctor of Philosophy in the Graduate School of Arts and Sciences, University of Pennsylvania. The author is now a member of the Department of Bacteriology and Parasitology of The University of Texas Medical Branch, Galveston. Received for publication January 20, 1952.

35° C. to 1/10–1/50 of the original volume. This product when injected intravenously into rabbits caused paralysis, diarrhoea and collapse, with death in 48 hours. A lethal dose (LD) was 0.1 ml. When injected intraperitoneally into guinea pigs, the toxic materials caused a fall in temperature and collapse. Again the LD was of the order of 0.1 ml. Autopsy of these animals showed congestion and hemorrhage of the intestines.

Todd (1904) observed this toxicity in 4–6 week old broth cultures. He considered it a soluble toxin which was highly active against rabbits and horses, while less active in guinea pigs, rats and mice. He brought out the interesting point that the organism of Flexner, *Shigella paradysenteriae* Flexner, did not form any such soluble toxin causing paralysis. This was the first indication that *S. dysenteriae* differed from the other organisms in this respect.

Vaillard and Dopter (1903) and Dopter (1905) used 24 hour broth cultures and found definite lesions in paralytic rabbits. The lesions in the spinal cord showed chromatolysis of the anterior horn cells, with small interstitial hemorrhages and focal necroses of the grey matter occurring frequently.

Kraus and Dorr (1905) made an important observation. They postulated from their data that there were two toxins produced: a soluble toxin, fatal to rabbits but not to guinea pigs which was present in 8–10 day broth or saline filtrates of 24 hour agar cultures and which gave rise to specific neutralizing antibody; and an insoluble toxin, present only in the cells, which was fatal to both rabbits and guinea pigs. The results obtained by Kraus and Dorr agreed with Todd (1904) that no soluble toxin could be found in *S. paradysenteriae* Flexner cultures.

Flexner and Sweet (1906) used toxin from 24 hour agar cultures in their studies with Shiga toxin. When they injected the toxic culture material intravenously into rabbits, it caused diarrhoea, paralysis, convulsions and death. The paralysis appeared in the front legs first, then extended to the hind limbs. Some animals lived as long as 10 days after the onset of paralysis, lying in a position termed opisthotonos. Pathological findings showed small hemorrhages in the brain and softening of the grey matter in the spinal cord. Intestinal pathology included congestion of the serosa, thickening of the walls, edema, psuedo-membranes

and hemorrhages, especially in the cecum. These workers suggested the possibility of two toxins, one neurotropic and the other intestinal. The neurotoxic factor was destroyed at 81° C. in 60 minutes.

Pfeiffer and Ungermann (1909) and Pfeiffer and Lubinski (1927) from their work with two different animal species, concluded that there were indeed two toxins: an exotoxin, causing neurotoxic symptoms in rabbits, and an endotoxin, which produced hypothermia and peritoneal exudation in guinea pigs.

Bessau (1911) agreed with previous theories on two toxins. He also indicated that one was a paretic (neurotropic) toxin attacking rabbits but not guinea pigs, while he called the other a marasmic (intestinal) toxin, active in both rabbits and guinea pigs. There were some early workers, among them Horimi (1913), who reported finding three toxins, the third one producing intestinal hyperemia in both rabbits and guinea pigs and additionally affecting the adrenal and mesenterial glands of the guinea pigs. A review of early work concerned with the relative activity and effectivity of antitoxic and antibacterial elements in anti-Shiga sera was presented by Lentz (1913).

Olitsky and Kligler (1920) performed some of the classical experiments with Shiga toxicity, which have been referred to in many subsequent studies. Their work showed the presence of two toxins when the organism was grown in broth for 3–4 weeks. These toxins could be separated and identified by their properties and their effects in animals. One was thermostable and was shown to be associated with the cellular substances of the organism, referred to as the endotoxin. It was the factor causing intestinal symptoms and was found to be the somatic antigen of the cell. The other toxic factor was present in the filtrate of younger broth cultures. It was heat labile and caused the paralytic or nervous symptoms in rabbits. The term neurotoxin was used to designate this exotoxic factor, for when it was injected intravenously into rabbits round cell infiltration was found in the brain and spinal cord. Sublethal doses caused transient paresis or paralysis of the extremities in 2–4 days, with preference for the anterior limbs. No intestinal lesions were observed.

McCartney and Olitsky (1923) extended the above observations by showing that the exotoxin (neurotoxin) could be found in 1–3 day broth filtrates, while the endotoxin was liberated only by autolysis later. They found that the exotoxin production was suppressed by anaerobic conditions. In animal experiments it was found that 1 of 5 rabbits developed neurotoxic symptoms when small collodion sacs containing two loopfuls of agar slant growth were placed intra-abdominally. They assumed formation of toxin by growth of the organism within the sacs while in the abdominal space.

Dumas, Ramon and Bilal (1926) showed that the toxicity of the Shiga cultures could be lowered by formaldehyde, as had been done previously with other bacterial exotoxins. This work led to many further investigations on the formation of toxoids for immunization purposes.

The work of Okell and Blake (1930) raised considerable controversy. They challenged the two-toxin theory championed by Olitsky and Kligler ten years previously. From their data they presented the theory that there was one toxin which was liberated only by autolysis. This factor they called an endotoxin. They did, however, suggest that other characteristics of the toxin were similar to classical exotoxins rather than endotoxins. It could be precipitated by 40 per cent ammonium sulfate which indicated its protein nature. The authors based much of their theory on the difference in reactivity of rabbits and guinea pigs to the toxic factor of the organism. These they considered to be inherent differences in animal susceptibility to the one toxin, not differences in the toxic moiety itself.

Barg (1932) reporting on the pathological effects of Shiga toxin also found that there was a reaction difference in rabbits and guinea pigs to the toxic factor, while Waaler (1936) agreed essentially with the one-toxin theory.

A new phase of investigation was begun by Kurauchi (1929). He employed chemical fractionation as a means of separating the components of the organism. He demostrated a specific polysaccharide derived from the cells which was active in the agglutinin and precipitin reactions. His work was confirmed by Meyer (1930, 1931) and Morgan (1931, 1932). Meyer and Morgan

(1935) found that this polysaccharide was capable of neutralizing hemolytic action of Shiga heterophile antibody.

Boivin and his associates, Mesrobeanu, Delaunay, Sarciron, Izard and Calalb (1936, 1937a–j, 1938a–c, 1940a,b) in prolific writings in the French literature have corroborated the two-toxin theory by means of chemical fractionation. Their method of separation consisted essentially of a trichloracetic acid precipitation of the neurotoxic protein. The somatic antigen, a complex molecule whose specificity was determined by a polysaccharide, remained in solution and could be purified by further chemical treatment. The *S* variants contained 10 per cent of the somatic antigen by dry weight. The *R* forms lacked this antigen. The polysaccharide could be split off by hydrolysis. Analysis showed that the polysaccharide contained 2–3 per cent nitrogen and represented 40–50 per cent of the complete antigen. Further studies indicated it was a hapten and the complete antigen consisted of a polysaccharide-lipid-fatty acid-protein complex.

Haas (1937, 1938, 1940) working independently in Germany agreed with the results of Boivin *et al.* as summarized above. He reported that the exotoxin was specific for the Shiga organism and could be obtained from both *R* and *S* variants. The endotoxin (somatic antigen) was similar to that of the other members of the *Shigella* genus as well as to that of the genus *Salmonella*, although no cross-reactions were noted in serological or animal protection tests.

Olitzki and Leibowitz (1935) and Olitzki, Benderesky and Koch (1943) attempted fractionation of *Shigella* antigens. The and Olitzki (1946) corroborated the finding that the thermofractions varying in toxicity and antigenicity. However, Koch and Olitski (1946) corroborated the finding that the thermolabile neurotoxin is present in both *R* and *S* variants, whereas they found the thermostable factor only in *S* cells.

Morgan and co-workers, Partridge and Schutze (1936, 1937, 1939, 1940, 1941, 1943) working independently in England obtained data similar to the above by using different methods of chemical fractionation and differential solubility with phenol, alkali, glycol and formamide. They were interested mainly in the endotoxic antigen of the cell but their results are quite important in comparing the chemical and biological (physiological

and antigenic) properties of their complete and degraded materials with the properties of the exo-neurotoxic factor, described by other investigators. Such a comparison throws much light upon the controversy of whether one or two toxins are produced by the Shiga organism. In summary, their data showed that the somatic antigen consisted of a complex of carbohydrate, lipid and protein. The carbohydrate was a specific hapten containing phosphorus. On degradation, the phosphorus could be split off and the carbohydrate solutions lost their viscosity and slight toxicity. The lipid was of the cephalin type, non-toxic and non-antigenic. The protein, in the undegraded form, contained a phosphorus prosthetic group and was antigenic. The removal of the prosthetic group removed antigenicity. The conjugated proteins of *Shigella dysenteriae* and *Salmonella typhosa* were found to be chemically and immunoligically indistinguishable. The protein material was only slightly toxic and apparently unrelated to any neurotoxin described by other workers. The carbohydrate-phospholipid complex was not antigenic, but the carbohydrate-conjugated protein showed antigenicity. The undegraded carbohydrate could be recombined with the conjugated protein and then became antigenic. The undegraded carbohydrate could combine with the simple protein but did not become antigenic, indicating that the phosphorus prosthetic group linkage was essential in the antigenicity. When the carbohydrate was absorbed onto collodion or to other proteins it did not regain antigenic properties, but in the undegraded form it could combine with the conjugated protein of the typhoid organism and become antigenic and specific for the Shiga organism. The *R* organisms contained no carbohydrate and their somatic antigen was only slightly antigenic even though similar to the *S* protein. The *R* and *S* cells contained at least 10 per cent of other proteins. The degraded carbohydrate did not recombine with conjugated protein. The Shiga carbohydrate induced typical passive anaphylaxis in guinea pigs and was related to the Shiga Forssman antigen which may be found in some strains of the organism. The Forssman antigen is found in no other *Shigella* species. Utilizing the complete somatic antigen, Morgan and his coworkers were able to produce antibodies in humans which

would cause agglutination of Shiga cells and would protect mice against 50 million viable cells suspended in mucin.

Istrati (1938), Steabben (1943) and Olitzki *et al.* (1943, 1946) agreed essentially with the work by Haas and others, stating that the neurotoxin was specific to Shiga and could be found both in *R* and *S* cultures. Olitzki declared that autolysis must take place to liberate it. Other workers, such as Takita (1939), had suggested autolysis as a means of getting maximum toxin in the "filtrate" of Shiga cultures. All of these observations stemmed from the original experiments of Conradi (1903) in his observations on the presence of toxin in autolysates.

Penner and Bernheim (1942) published results of their work with Shiga toxic substances in animals. They described pathological changes following the injection of autolysates of whole cultures. The animals showed the multiplicity of symptoms and pathology which one would expect from mixtures of bacterial substances. Their conclusions should be considered in this light.

Olitzki and Koch (1943) summarized some of the bacterial nutrition investigations of Doerr, who found no toxin in synthetic media, and Cohnbronner, who found slight toxicity using ammonium as a source of nitrogen and lactate as a source of carbon. The latter, however, showed that 1 to 2 ml. of filtrate was necessary to kill animals. Olitzki and Koch found very little toxin in this medium. In later nutritional work, Olitzki and Bichowsky (1946) produced toxin in a buffered casein hydrolysate medium. Their medium yielded toxicity at a 1:50 dilution. Farrell (1943) suggested pork infusion as a stimulant for toxin production. Dubos (1946) developed a peptone medium free of material precipitable at pH 4, and from which all free iron had been removed. He reported that iron would prevent toxin formation as had been shown previously with diphtheria and tetanus toxins by Pappenheimer and Johnson (1936) and Mueller and Miller (1940).

Following the report of Dumas and co-workers (1926) as noted above, many workers became interested in toxoids for immunization purposes, because they felt that the disease could be attacked by antitoxic antibodies in a similar fashion to diphtheria or tetanus. Okell and Blake (1930), Olitzki *et al.* (1943,

1946), Anderson *et al.* (1945), Farrell, Fraser and Ferguson (1944), Steabben (1943) and Dubos (1946), all utilized formaldehyde as a means of detoxification. Each had slight modifications as to per cent formaldehyde, temperature and time, but they all reached the same conclusions, that formaldehyde could detoxify while maintaining the antigenicity of the material. Treffers (1946) resorted to acetic anhydride for acetylation of dysentery antigens. Olitsky and Kligler (1920) and Waaler (1936) found that a temperature of 80° C. would reduce toxicity and maintain antigenicity. Anderson and co-workers (1945) reported hexamethylenetetramine as a substitute for formaldehyde. Branham and Habel (1946) experimented with ultraviolet radiation. Their data indicated the toxin to be quite resistant to such detoxification procedure.

The role of the toxin in infection is a debated point. A review of early work concerned with the relative activity and effectivity of antitoxic and antibacterial elements in anti-Shiga sera was presented by Lentz (1913). In early protection tests in animals, Okell and Blake (1930), observed that both bacillary bodies and filtrates gave the same antitoxin and protected against each other. Waaler (1936) agreed with this, but more recent work with chemically separated components has yielded somewhat different results. Steabben (1943), for example, demonstrated that antibacterial sera are protective against infection but not against the toxin. Antitoxic sera did not protect against infection. Antisera to the *R* organism protected against *R* organisms only. Olitzki *et al.* (1947) likewise reported that antibacterial serum while it would free the blood of bacteria and modify the infection, would not prevent intoxication.

Branham (1948), on the other hand, feels that antitoxic antisera are necessary in the treatment of the disease because of the differences in toxin reported by earlier workers and the differences in protection from antisera against the somatic antigen and the neurotoxin. Weil (1947), however, in a review article, reports on the failure of toxoid prophylaxis in clinical trials.

Pappenheimer (1948) considers the Shiga neurotoxic factor to be an endotoxin because it is not present in culture filtrates in the same quantities as diphtheria or botulinus or tetanus

toxins. He does, however, feel that in all other characteristics it is more like an exotoxin.

In two papers, summarizing his work on the toxins and antigens of *Shigella dysenteriae*, Boroff (1949) reports his inability to find evidence for the existence of an exotoxin. He considers that all the toxic properties are due to a single endotoxin, which is also the dominant antigen. Tal (1950) points out, however, that Boroff's somatic antigen differs markedly in toxicity from that found by others (Koch and Olitzki, 1946). It seems likely that his failure to find a separate and distinct exotoxin may be occasioned by incomplete separation of the toxic factors rather than by actual absence of a neurotoxin.

It would appear that there are questionable and debatable points surrounding the formation of Shiga neurotoxin *in vitro* and *in vivo*. There are controversial issues over whether or not the neurotoxin and endotoxin are distinct and separate entities. Little information can be found on the biochemical properties and other characteristics of the neurotoxin itself. Certain questions concerning the effect on the toxin of physical and chemical factors remain to be answered. Some doubt has arisen as to the role of the toxin in the disease process in both animals and humans.

It is hoped that articles to be published, which summarize our experimental work, may bring out new data to settle some of the controversies by clarifying certain issues and substantiating previous findings, with particular reference to the formation of the neurotoxic factor *in vitro*, characterization of the neurotoxin biologically and biochemically, and elucidation of its role in animal and human infections and intoxications.

BIBLIOGRAPHY

Anderson, C. G., Brown, A. M., and Macsween, J. C.: The production, purification and titration of the neurotoxin of *Shigella dysenteriae*. *Brit. J. Exptl. Path.*, 26, 197–208, 1945.

Ando, K.: A simple method of obtaining specific soluble substance from various bacteria (*Streptococcus viridans*, *B. dysenteriae* and *B. mallei*) (preliminary report). *J. Immunol.*, 17, 555–557, 1929.

Barg, G. S.: Zur Charakteristic der Wirkung des Dysenterietoxins, *Z. Immunitatsforsch.*, 74, 372–379, 1932.

Bessau, G.: Ueber die Dysenteriegift und ihre Antikorper. Zentr. Bakt. Parasitenk., *I. Orig.*, 57, 27–56, 1911.

Boivin, A.: Sur le comportement compare des entotoxines et des exotoxines vis-a-vis de l'acide trichloroacetique. *Compt. rend. soc. biol.*, 203, 284–286, 1936.

Boivin, A., and Izard, Y.: Methode pour la purification a l'acide trichloracetique, des toxines et anatoxines diphtheriques, tetaniques et staphylococciques. *Compt. rend. soc. biol.*, 124, 25–28. 1937a.

Boivin, A., and Mesrobeanu, L.: Recherches sur la nature chimique et sur les proprietes biologiques, des antigenes somatiques et des toxines des bacteries. *6th Congre de Chimie Biologique*, 401–413, 1937b.

Boivin, A., and Mesrobeanu, L.: Recherches sur les toxines des bacilles dysenteriques. Sur la nature et sur les proprietes biologiques des principes toxiques susceptible de se rencontrer dans les filtrats des cultures sur bouillion du bacille de Shiga. *Compt. rend. soc. biol.*, 124, 442–444, 1937c.

Boivin, A., et Mesrobeanu, L.: Recherches sur les toxines des bacilles dysenteriques. Sur les principes toxiques du bacille de Flexner. *Compt. rend. soc. biol.*, 124, 1078–1080, 1937d.

Boivin, A., et Mesrobeanu, L.: Sur le pouvoir protecteur antitoxique des serums obtenus en infectant a l'animal des endotoxines glucidolipidiques. *Compt. rend. soc. biol.*, 124, 1092–1094, 1937e.

Boivin, A., et Mesrobeanu, L.: Recherches sur les toxines des bacilles dysenteriques; sur le pouvoir protecteur antitoxique des serums obtenus en injectant a l'animal l'endotoxine-antigene O du bacille de Shiga et du bacille de Flexner. *Compt. rend. soc. biol.*, 125, 796–798, 1937f.

Boivin, A., et Mesrobeanu, L.: Recherches sur les toxines des bacilles dysenteriques. Sur l'existence d'un principe toxique thermolabile et neurotrope dans les corps bacteriennes du bacille de Shiga. *Compt. rend. soc. biol.*, 126, 222–224, 1937g.

Boivin, A., et Mesrobeanu, L.: Recherches sur les toxines des bacilles dysenteriques; sur l'indentite entre la toxine thermolabile et neurotrope des corps bacteriennes du bacille de Shiga et l'exotoxine present dans les filtrats des cultures sur bouillion de la meme bacterie. *Compt. rend. soc. biol.*, 126, 323–325, 1937h.

Boivin, A., et Mesrobeanu, L.: Recherches sur les toxines du bacille dysenterique. Sur la signification des toxines produits par le bacille de Shiga et par le bacille de Flexner. *Compt. rend. soc. biol.*, 126, 652–655, 1937i.

Boivin, A., et Mesrobeanu, L.: Sur l'existence de toxine thremolabile et neurotrope (exotoxine) dans les corps bacteriennes du bacille de Shiga. *Compt. rend. acad. science*, 204, 1759–1761, 1937j.

Boivin, A., Mesrobeanu, L., et Izard, Y.: Sur la separation, par voie chimique, des endotoxines glucidolipidiques et des exotoxines proteiques. *Compt. rend. soc. biol.*, 127, 651–654, 1938a.

Boivin, A., and Mesrobeanu, L.: Sur la specificite qui se manifeste dans l'action immunisants anti-infecteuse des antigenes glucido-lipidiques. *Compt. rend. sc. biol.*, 128, 358–360, 1938b.

Boivin, A., et Mesrobeanu, L.: Recherches sur les toxines des bacilles dysenteriques. Sur les rapports qui existent entre la presence d'antigene glucido-lipidique chez le bacille de Shiga et la virulence et le pouvoir vaccinant de cette bacterie. *Compt. rend. soc. biol.*, 128, 446–448, 1938c.

Boivin, A.: Les deux toxines du bacille de Shiga et leur places dans la classification general des toxines bacteriennes. *Rev. Immunol.*, 6, 86–115, 1940a.

Boivin, A., Delaunay, A., and Sarciron, R.: Sur la liberation de l'antigene glucido-lipidique et de la neurotoxine par les cellules mortes du bacille de Shiga. *Compt. rend. soc. biol.*, 134, 361–363, 1940b.

Boroff, D. A.: Study on toxins and antigens of *Shigella dysenterie*. I. Toxicity and antigenicity of whole organisms and various fractions of *Shigella dysenteriae*. *J. Bact.*, 57, 617–632, 1949.

Boroff, D. A., and Macri, B. P.: Study on toxins and antigens of *Shigella dysenteriae*. II. Active protection of rabbits with whole organisms and fractions of *Shigella dysenteriae*. *J. Bact.*, 58, 387–394, 1949.

Branham, S. E., and Habel, K.: Preparation and evaluation of an irradiated toxoid from the toxin of *Shigella dysenteriae*. *J. Immunol.*, 54, 305–314, 1946.

Branham, S. E., and Carlin, S. A.: Studies with *Shigella dysenteriae* (Shiga). I. Infection and toxin action in mice. *J. Infectious Diseases*, 83, 60–65, 1948.

Branham, S. E., and Carlin, S. A.: Studies with *Shigella dysenteriae* (Shiga). II. The effect of serum on experimental infection and intoxication in mice. *J. Infectious Dis.*, 83, 66–77, 1948.

Chantemesse, A., and Widal, F.: Le microbe de la dysenterie epidemique. *La semaine Med.*, 8, 153, 1888.

Conradi, H.: Ueber losliche, durch aseptique Autolyse erhaltene Giftstoffe von Ruhr- und Typhus bacillus. *Deutsch Med. Wochenschr.*, 29, 26–28, 1903.

Dopter, C.: Effets experimentaux de la toxine dysenterique sur le systeme nerveux. *Ann. Inst. Pasteur*, 19, 353–366, 1905.

Dopter, C.: Effets experimentaux de la toxine dysenterique sur le systeme nerveux central. *Compt. rend. soc. biol.*, 58, 400–402, 1905.

Dubos, R. J.: The bacterial cell. Harvard University Press, Cambridge, Mass., 1945.

Dubos, R. J., and Geiger, J. W.: Preparation and properties of Shiga toxin and toxoid. *J. Exptl. Med.*, 84, 143–156, 1946.

Dumas, J., Ramon, G., and Bilal, S.: Anatoxine dysenterique. *Ann. Institt. Pasteur*, 40, 134, 1926.

Farrell, L., and Ferguson, H.: Shiga toxoid. *Canad. J. Pub. Health*, 34, 130–139, 1943.

Farrell, L., Fraser, D. T., and Ferguson, H.: Trial of dysentery toxoid (Shiga) in human volunteers. *Canad. J. Pub. Health*, 35, 311–316, 1944.

Felsen, J.: *Bacillary dysentery, colitis and enteritis.* Saunders, Philadelphia, Pa., 1945.

Flexner, S.: On the etiology of tropical dysentery. *Bull. Johns Hopkins Hospital*, 11, 231–242, 1900.

Flexner, S., and Sweet, J. E.: The pathogenesis of experimental colitis and the relation of colitis in the animals and in man. *J. Exptl. Med.*, 8, 514–535, 1906.

Grigor'eff, A. W.: Zur Frage der Mikroorganism bei Dysenterie. *Zentr. Bakt. Parasitenk., Ref.*, 876, 12, 1892.

Haas, R.: Uber Endo- und Exotoxine von Shiga-bazillen. *Z. Immunitatsforsch.*, 91, 254–272, 1937.

Haas, R.: Uber Exo- und Endotoxine der Dysenteriebazillen. II. Weitere Studien uber Endotoxine von Shiga und Flexner Ruhrbazillen. *Z. Immunitatsforsch.*, 92, 355–381, 1938a.

Haas, R.: Uber Endo- und Exotoxine von Ruhrbrazillen. III. Vorkommen und Eigenschaften eines Endotoxines der E-Ruhrbrazillen (Kruse-Sonne). *Z. Immunitatsforsch.*, 94, 239–246, 1938b.

Haas, R.: Uber Exo- und Endotoxine von Dysenteriebazillen. IV. Beitrag sur Kenntnis der Endotoxine der Ruhrbazillen. *Z. Immunitatsforsch.*, 94, 480–488, 1938c.

Haas, R.: Vergleichende Untersuchunger uber die toxischen und antigenen Eigenschaften von bei tiefer Temperatur gewonnen lislichen Bakterienbestanteilen der Typhus-Paratyphus-Enteritis und Ruhr-gruppe zugleich. 5. Mitteilung der Untersuchungsreihe uber Endo- und Exotoxine von Dysenterbazillen. *Z. Immunitatsforsch.*, 97, 317–329, 1940a.

Haas, R.: Untersuchungen zur Kenntnis des Exotoxins und Formol-toxoids von *Bact. dysenterie* Kruse-Shiga. 6. Mittelung uber Endo- und Exotoxins von Dysenteriebakterien. *Z. Immunitatsforsch.*, 99, 121–141, 1940b.

Horimi, K.: Ueber die Pathogen wirkungen der Dysenterietoxine. Centr. Bakt., *1 Abt., Orig.*, 68, 342–358, 1913.

Istrati, G.: Action neurotrope et enterotrope de la toxine proteique thermolabile du bacille de Shiga. Action enterotrope de l'antigene glucidolipidique du bacille de Shiga "S." *Compt. rend. soc. biol.*, 129, 1010–1013, 1938.

Koch, P. K., and Olitzki, L.: The action of dysentery toxins on different laboratory animals. *Exptl. Med. Surg.* 4, 54–68, 1946.

Kraus, R., and Dörr, R.: Ueber experimentelle Therapie der Dysenterie. *Wiener Klinisch Wochenschrift*, 18, 1077–1079, 1905.

Kruse, W.: Ueber die Ruhr als Volkskrankheit und ihrer Erreger. *Deutsche Med. Wochenschrift*, 26, 637–639, 1900.

Kurauchi (see Ando, K.)

Lentz, O.: Die dysenteriebacillen in Kolle-Wasserman, Handbuch d. Pathogenen Mikroorganismen, 2-Aufl., G. Fischer, *Jena*, 3, 899–914, 1913.

McCartney, J. F., and Olitsky, P. K.: Separation of the toxins of *Bacillus dysenteriae Shiga, J. Exptl. Med.* 37, 767–779, 1923.

Meyer, K.: Zur chemischen Natur des heterogenetischen Antigens in Shiga-Bazillen. *Z. Immunitatsforsch.*, 68, 98–108, 1930.

Meyer, K.: Zur Chemischen Natur des heterogenetischen Antigens in Shiga-Bazillen. *Z. Immunitatsforsch.*, 69, 499, 1931.

Meyer, K. F., and Morgan, W. T. J.: Relationship between heterophile hapten and specific polysaccharide hapten of "smooth" form of *Bact. Shigae. Brit. J. Exptl. Path.*, 16, 476, 1935.

Morgan, W. T. J.: A specific precipitating polysaccharide from *B. dysenteriae* (Shiga). *Brit. J. Exptl. Path.*, 12, 62–66, 1931.

Morgan, W. T. J.: The estimation of small amounts of a specific bacterial polysaccharide by induction of anaphylaxis. *Brit. J. Exptl. Path.*, 13, 342–349, 1932.

Morgan, W. T. J.: Studies in immunochemistry; preparation and properties of specific polysaccharide from *B. dysenteriae* (Shiga). *Biochem. J.* 30, 909–925, 1936.

Morgan W. T. J.: Studies in immunochemistry. II. The isolation and properties of a specific antigenic substance from *B. dysenteriae* (Shiga). *Biochem. J.*, 31, 2003–2021, 1937.

Morgan, W. T. J., and Partridge, S. M.: Bacterial antigens. *Nature*, 143, 1025–1026, 1939.

Morgan, W. T. J., and Partridge, S. M.: Studies in immunochemistry. 4. The fractionation and nature of antigenic material isolated from *Bact. dysenteriae* (Shiga). *Biochem. J.*, 34, 169–191, 1940.

Morgan, W. T. J., and Partridge, S. M.: Studies in immunochemistry. Use of phenol and of alkali in degradation of antigenic material. *Biochem. J.*, 35, 1140–1163, 1941.

Morgan, W. T. J., and Schutze, H.: Prophylactic inoculation with the O antigen of *Bact. Shigae. Lancet*, ii, 284, 1943.

Mueller, J. H., and Miller, P. A.: Tetanus toxin production on a simplified medium. *Proc. Soc. Exptl. Biol. Med.*, 43, 389–390, 1940.

Okell, C. C., and Blake, A. U.: Dysentery toxin (Shiga): notes on its preparation with a discussion of its position as an endotoxin. *J. Path. Bact.*, 33, 57–63, 1930.

Olitsky, P. K., and Kligler, I. J.: Toxins and antitoxins of *Bacillus dysenteriae* Shiga. *J. Exptl. Med.*, 31, 19–33, 1920.

Olitzki, L., Benderesky, J., and Koch, P. K.: Studies on the toxins of *Shigella dysenteriae* (Shiga). *J. Immunol.*, 46, 71–82, 1943.

Olitzki, L., and Koch, P. K.: Production of potent toxins by *Shigella dysenteriae* (Shiga) in a synthetic medium. *Nature*, 151, 334, 1943.

Olitzki, L., and Bichowsky, L.: The preparation of a potent toxin of Shigella dysenteriae (Shiga) on a semi-synthetic medium and its use for the preparation of an alum-precipitated toxoid. *J. Immunol.*, 52, 293–300, 1946.

Olitzki, L., and Koch, P. K., and Shelubsky, M.: Experimental infection of mice with *Shigella dysenteriae* and modification of the infection by means of antitoxic and antibacterial sera. *Exp. Med. Surg.*, 7, 206–218, 1947.

Olitzki, L., Shelubsky, M., and Koch, P. K.: A thermolabile substance of *Shigella dysenteriae* Shiga. *J. Hyg.*, Camb., 44, 271–275, 1946.

Pappenheimer, A. M., Jr., and Johnson, S. J.: Studies in diphtheria toxin production. I. The effect of iron and copper. *Brit. J. Exptl. Path.*, 17, 335–341, 1936.

Pappenheimer, A. M., Jr.: Proteins of pathogenic bacteria. *Advances in protein chemistry*, IV, 123–153, 1948.

Penner, A., and Bernheim, A. I.: Studies in the pathogenesis of experimental dysentery intoxication. *J. Exptl. Med.*, 76, 271–282, 1942.

Pfeiffer, R., and Ungermann, E.: Zur antitoxin Frage bei der dysenterie. *Centr. Bakt.*, 1. Abt., Orig., 50, 534–541, 1909.

Pfeiffer, R., and Lubinski, H.: Ueber die wirkung des formalins auf Endotoxin. *Centr. Bakt.*, 1. Abt., Orig., 102, 459–470, 1927.

Pillemer, L., and Robbins, K. C.: Chemistry of toxins. *Ann. Rev. Microbiol.*, III, 265–288, 1949.

Shiga, K.: Ueber den Erreger der Dysenterie in Japan. *Zentr. Bakt. Parasitenk.*, I. Abt., 23, 599–600, 1898.

Shiga, K.: Ueber die Dysenteriebacillus *(Bacillus dysenteriae)*. *Zentr. Bakt. Parasitenk.*, I Abt., 24, 817–824, 1898.

Steabben, D.: A study on bacteriological lines of the antigens derived from *Bact. dysenteriae* and of their antisera in protective tests against the living organisms. *J. Hyg.*, 43, 83–95, 1943.

Takita, J.: Production of a strong toxin of the Shiga dysentery bacillus by bouillion cultures. *Kitasato Arch. Exptl. Med.*, 16, 174–181, 1939.

Tal, C.: Differences in toxicity of the S- and R- variants of *Shigella dysenteriae*. *J. Immunol.*, 65, 221–227, 1950.

Todd, C.: On a dysentery toxin and antitoxin. *J. Hyg.*, 4, 480–494, 1904.

Topley, W. W. C., and Wilson, G. S.: *Principles of bacteriology and immunology*. 3rd edition, The Williams and Wilkins Co., Baltimore, Md., 1946.

Treffers, H. P.: The detoxification by acetylation of soluble antigens from *Shigella dysenteriae* (Shiga) and E. typhosa. Science, 103, 387–389, 1946.

Treffers, H. P.: The detoxification of dysentery and typhoid-soluble antigens by chemical means. *J. Bact.*, 51, 642, 1946.

Vaillard, L., and Dopter, C.: La dysenterie epidemique. *Ann. inst. Pasteur*, 17, 463–491, 1903.

Waaler, E.: Studies on the toxin production of the Shiga bacilli. *J. Exptl. Med.*, 63, 1–15, 1936.

Weil, A. J.: Progress in the study of bacillary dysentery. *J. Immunol.*, 46, 13–46, 1943.

Weil, A. J.: Medical and epidemiological aspects of enteric infection. *Ann. Rev. Microbiol.*, 1, 309–332, 1947a.

Weil, A. J.: Dysentery—a progress report for the years 1942–1946. *J. Immunol.*, 55, 363–405, 1947b.

ALTERATION OF THE BLOOD PICTURE BY SECONDARY X-RADIATION*

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The widespread use of ionizing radiations in biological research necessitates the employment of proper personnel safety precautions. Although the dangers of direct radiations are generally understood, the widespread and often repeated X-irradiation of pregnant women would indicate a dangerous laxity in the use of such equipment. Equally great, and perhaps potentially greater, are the dangers which may result from secondary radiations emanating, not only from the instruments, but from the direct beam of X-radiation by reflection, since adequate safeguards against these may not be provided, inasmuch as the dangers may not be recognized.

Even though the intensity of the secondary radiation may be low, the effect is cumulative, since the total dose is a function of intensity and time. As Muller (1950) states, "The frequency of the mutations induced by the radiation depends upon the total exposure and not upon how the dose was distributed in time. This is to say, a given dose applied in the form of a very short, intense treatment will induce the same number of mutations as the same total dose when it is divided into a large number of parts, even with long intervals beween them, or when it is applied in the form of a very protracted treatment of extremely low intensity."

It is obvious that the ubiquitous X-ray machine is a potential source of unsuspected danger.

The effects upon the peripheral blood picture following total body irradiation with primary X-radiation are fairly well understood. Price (1951) reports that blood counts offer a reliable method for gauging the damage resulting from various radiation dosages, although the work of Furth, *et al.* (1951)

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indicates that high doses (600–800 r) alter the volume relationships of the plasma, so that direct counting techniques do not give a true picture of the total numbers of cells present. Doses between 300 and 400 r, however, do not change the volume relationships (Supplee, *et al.* 1951). For this reason dosages within the latter range were used in our experiments.

Materials and Methods

Young male Swiss albino mice weighing approximately 20 grams each were given uniform individual total body radiation dosages in plastic tubes. Factors of irradiation were: 85 kvp; 4 ma; TSD, 54 cm; filtration, 1.75 mm aluminum; 1.5 mm lead diaphragm with 2.9 x 2.9 cm aperature. The dosage rates, measured with a 100 r Victoreen Condensor r-meter, were approximately 8.0 r/min for primary radiation, and 6.5 r/min for secondary radiation experiments. The maximum variability in dose delivered to individual mice in any case was 2.1%. The average surface dose of primary radiation was 326 r, while the dose of secondary radiation was 322 r. A fluorescent screen was used to aid in aiming the beams and for determining limits of the primary radiation field. Only the homogeneous center portion of the beam, as determined by measurement, was used. Most of the undesirable secondary radiation was eliminated in the primary radiation experiments by suspending the mice "in air" upon thin cardboard. Secondary radiation was produced by introducing a masonite pressed wood phantom into the primary beam. The mice were placed completely outside the primary beam and at right angles to it. The animals sometimes changed positions slightly in the plastic tubes, but due to their small body size this did not alter the integral dose appreciably, and it is also believed that the decrease in depth-dose with such small animals was not of critical importance.

Blood was collected at 24-hour intervals from the snipped tail, and hematological counts were made in the standard manner.

Experimental Results

Erythrocyte and leukocyte counts were averaged from 198 determinations, made at 24-hour intervals over a four-day pre-irradiation period. The average erythrocyte and leukocyte counts for these animals were 8,709,000 ± 150,000 and 17,054 ± 550, respectively. The leukocyte count is somewhat higher than normal due undoubtedly to the fact that the tail-snip procedure was used for collecting blood, although aseptic techniques were used, and no tail infections were noted.

The hematological picture resulting from primary radiation (Fig. 1) was typical, showing a rapid drop within a short time after treatment. Animals surviving 12 days (83%) recovered. Differential counts indicated that curves for lymphocytes and neutrophiles closely followed the total leukocyte curve.

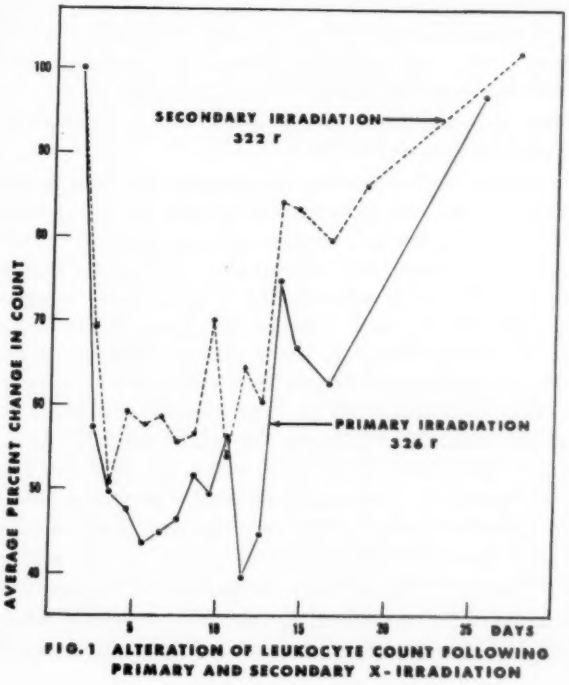

FIG. 1 ALTERATION OF LEUKOCYTE COUNT FOLLOWING PRIMARY AND SECONDARY X-IRRADIATION

As may be observed from Figure 1, the blood picture following secondary irradiation is generally similar to that resulting from primary radiation treatment. The slightly higher dose of primary radiation used accounts for the greater effect upon total leukocytes. Erythrocyte counts appear to decrease slowly following either type of irradiation at a rate of about one per cent per day.

Conclusions and Summary

A method of irradiating small animals by "suspending them in air," has been employed. This was accomplished by using a thin layer of cardboard to support the plastic tubes. The cardboard has approximately the same average atomic weight as air, and hence emits similar secondary radiations.

These experiments demonstrate that the same gross hematological picture results following either primary or secondary irradiation. The only difference lies in the fact that exposure to secondary radiation usually is of a low order, but is of such a nature that exposures are frequent and intermittent over relatively long periods of time.

One cannot overemphasize the dangers involved in the use of ionizing radiations. It is extremely easy for the investigator or practitioner to become complacent, especially to the secondary radiation, when he is continually working with it. Small repeated doses of even a few tenths of a roentgen, being cumulative, soon add up to a considerable does. If the individual is still of reproductive age, the resulting damage (mutations, decrease in viability and longevity of sperm) to his gonads will very likely manifest itself in later generations. Even though he has no additional offspring, the total dose may materially affect his own body in devious ways, possibly resulting in somatic mutations, increased propensity to cancer, etc. Protective equipment, such as lead-impregnated gloves and aprons, is often of little real value and may, by giving the user a false sense of security, be actually dangerous. It is essential that these dangers be recognized by everyone working with high energy radiations. All radiation and protective equipment

should be thoroughly tested to meet rigid safety standards prior to use.

LITERATURE CITED

Furth, J., G. A. Andrews, R. H. Storey and L. Wish. 1951. The effect of X-irradiation on erythrogenesis, plasma, and cell volumes. *Oak Ridge Natl. Lab. ORNL-878.*

Hunter, F. T., O. E. Merrill, J. G. Trump and L. L. Robbins. 1949. The protection of personnel engaged in roentgenology and radiology. *New Eng. J. Med.* 241:79.

Muller, H. J. 1950. Radiation damage to the genetic material. *Am. Sci.* 38:33–59, 126.

Muller, H. J. 1950. Radiation damage to the genetic material. II. Effects manifested mainly in the exposed individuals. *Am. Sci.* 38:399–425.

Price, C. H. G. 1951. Observations upon the lymphopenia of X-ray irradiation. Part II. *Brit. J. Radiology* 24:556–67.

Supplee, H., J. D. Hauschildt and C. Entenman. 1951. Plasma proteins and plasma volumes in rats following total body X-irradiation. *Naval Radiolog. Def. Lab. AD–318 (B).*

THE ROLE OF MEDICINE IN THE CARE OF THE ALCOHOLIC*

JOSEPH HIRSH†

Introduction

Medicine has traditionally taken a dim view of the alcoholic —and with good reason. He has earned for himself the unenviable reputation of being a contumacious nuisance, a disruptive force in private practice and, until relatively recently, an unredeemably hopeless patient. The physician has, perforce, viewed the alcoholic with the pessimism of repeated failures in treatment, and been conditioned by the repetitive nature of relapses and the knowledge that *no effective specific therapy* existed. (And most physicians have always viewed multitherapy—the method in handling the alcoholic—with skepticism.)

The hard but undeniable fact is that alcoholism could not in the past and cannot at present be cured, at least in the sense that other diseases are cured. ACTH, ACE, antabuse, emetine, psycho- and vitamin-therapy notwithstanding, there is no more specific cure for alcoholism today than there is for tuberculosis or cancer. Yet the absence of specific therapies for these other diseases has not deterred physicians from treatment in the hope of achieving *recovery* and *rehabilitation*—goals which are attainable and have been attained for thousands of alcoholics when the resources of medicine have been made available to them.

Most practitioners' views of the alcoholic have been conditioned on the one hand by failure and, on the other, by the assumption that they knew all there was to be known about the alcoholic who, afer all, was a simple characterological problem. Of course, nothing could be farther from the truth. Only

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now, after years of research, are we beginning to have some idea of the dynamics of alcoholism. What we have learned fully justifies the view that the alcoholic is as much the responsibility of the physician as the cancer patient. And clinical experience, over the past decade in particular, fortifies the conviction that medicine can redeem and rehabilitate into productive citizens thousands of patients once abandoned as hopeless.

The Attitude of the Patient

The receptivity of the patient to medical care is generally conceded to be an essential for successful treatment. The prognosis for treatment initiated not for himself but to satisfy someone else is poor. Unless the patient acknowledges that his life is becoming or has become unmanageable through his recourse to, and dependence upon, alcohol the resources of medicine are to little avail beyond traditional sobering up treatment and supportive therapy—roles most physicians understandably abjure because they are, measured in terms of long-range goals, profitless and wasteful.

Unless the patient really wants to be helped and makes an effort at the initiation of treatment to stop drinking and maintain sobriety, treatment will almost surely fail. Medically, as we shall discuss later, he can be helped to maintain sobriety. He should not be permitted, however, to lean on the current crutch for some alcoholics, namely, that *alcoholism is a sickness*, and play a passive role. Unless the patient maintains an active interest and role in treatment, the outlook is not good. His desire to recover, to be rehabilitated, is all-important.

Another important element in the psychology of the patient—one which can be used as a constant and powerful instrument in therapy—is the fact that, in coming to the physician for care, something (possibly for the first time in his experience) is really going to be done to him and for him. The very fact that the physician is sufficiently interested to consider him a patient instead of an object of moral turpitude is in itself an important psychologic factor in therapy and points up the need for a sympathetic approach to him.

The Attitude of the Physician

For many alcoholics the whole process of living is a difficult and complicated one; it is frequently the reason why they become alcoholics in the first place. This fact must be constantly borne in mind during therapy and calls for the very best in medicine in the way of patience, compassion, and understanding. It calls for the same sufferance and understanding towards recidivism as to recurrences of other psychologic or somatic ailments. It demands that the goals set for the patient in therapy—whether they be abstinence, adequate diet, new interpersonal and group relations, new recreational activities, or vocational pursuits—be spaced one at a time, rarely two or more in combination. If the problems in therapy become too great for the patient, he will more often than not abandon it.

It has been said that alcoholism is an example par excellence of the "vicious circle" and where, when and how to break in on it may indeed tax one's ingenuity. One cannot wait, however, for *the* propitious moment for it may never come. Alcoholic patients do and will come into the doctor's office acutely intoxicated, hung-over, as well as during periods of relative or complete sobriety. Their medical management during acute intoxication has been discussed by Fox.

Ordinarily, treatment of their alcoholism while acutely intoxicated is considered impossible. Observation of patient behavior under the influence of alcohol, however, often provides useful clues for later understanding of the psychodynamics involved. Acceptance of the patient *as a patient* rather than as a "drunk" often constitutes a first step towards rehabilitation; certainly it is *the* first step in establishing that necessary rapport between doctor and patient which is central to the successful treatment of the alcoholic.

Important as is the management of the acutely intoxicated patient, the patient who has been on an alcoholic bout poses some special problems for the practitioner. He is tremulous, restless, irritable, apprehensive and sleepless. He is often depressed, guilt-ridden and in a state of emotional decompensation. He has the "jitters" and may develop audio-visual hallucinations. In all likelihood he has eaten little or nothing for several

days or weeks, is revulsed by food, has mild to severe gastrointestinal disturbances and is dehydrated. In short, he manifests many of the characteristics common to anxiety tension states.

If during this period of hopelessness and helplessness, condemnation and rejection come from his doctor, he may turn further in to himself; he may try to drink again or even to kill himself. On the other hand, if he finds real understanding and sympathy, real help for his physical pain and genuine interest in his problems, the flood gates of emotion may open and information and contentful material that ordinarily during sober periods might take many sessions to elicit, will come pouring out. Fox points out, "Along with reassurance, a few questions about past life and experiences may connect the present emotions with some long forgotten similar ones and a few interpretations now may facilitate deeper understanding of the basic conflicts later on."

During the hangover state the patient should be seen daily to cement early physical and emotional gains. With each passing day he will want to forget his recent helplessness and in fact deny it and with it the problem of alcoholism. The daily session tends to strengthen his acceptance as a patient—and even more —as a worthwhile person, giving him courage to face himself, perhaps for the first time.

The Role of the Physician in Treatment

When the patient has finally accepted the fact that he needs help and wants it, the type or, as is more often the case, the types of treatment must be decided upon. Physical rehabilitation, obvious as it is, is an essential first step. The existence of subacute, acute, and chronic conditions common to many alcoholics must be corrected not only as intrinsic problems but also because these patients rationalize the presence of pain, fatigue, debility, and insomnia to justify their recidivism. The correction of these somatic problems—the gastrointestinal disturbances, the malnutrition and anemia, the vitamin deficiency diseases—is not only a prerequisite to dealing with the personality problems of the patient but in itself makes a surprising difference in the character and content of these problems.

Early in the treatment of somatic problems, assessment should be made* of the patient's personality liabilities and strengths. For sound somatic reasons and to permit early personality assessment and the institution of psycho-therapy, abstinence is among the first orders of business. There are few, if any, contraindications to immediate and complete withdrawal.

As part of the personality assessment, evaluation of psychosocial factors predisposing to or precipitating excessive drinking is essential. This can be done solely by interview. We have found a questionnaire completed by the patient at home, following his initial visits, to be exceptionally useful. This procedure is saving of the physician's time but must be followed up by interviews to complete the data, to amend mistaken impressions submitted in response to the questionnaire, and to interpret adequately hidden or fraudulent material.

Physical evaluation of the patient is done coextensively with psychosocial evaluation. At the Alcoholics Treatment Center the usual physical examination includes neurological study, chest X-ray, gynecological and rectal examination, search for vitamin deficiency disease and liver dysfunction. Laboratory tests are made as indicated and EKG is routinely done if Antabuse is to be prescribed.

Since Antabuse (TETD) is now available to the medical profession at large and is one of the most effective ways of breaking into as well as breaking up the "vicious circle," a discussion of this drug is in order.

Tetraethylthiuram Disulfide (TETD or Antabuse) Treatment

The peculiar property of rendering anyone taking TETD extremely sensitive to alcoholic beverages was discovered accidently four years ago in Denmark. Since then a great deal has been learned concerning its action and effectiveness. It has been used on tens of thousands of cases in Europe, Canada and the United States. After three years of intensive research covering

*One of the most rapid and reasonably useful ways is to refer the patient to a clinical psychologist for testing and evaluation. The basic tests we use are Rorschach, Bellevue-Wechsler, Word Association and Figure Drawing.

12,000 cases at 100 investigative centers in this country, TETD was recently released for prescription use by the Pure Food and Drug Administration.

TETD or *Antabuse* (Ayerst, McKenna and Harrison Ltd.), derived from the two words *anti* and *abuse*, is now available in tablet form of .5 gm. each. Administered with the appropriate precautions and in proper doses it is a harmless drug. In over 12,000 patients in the U.S. and Canada to whom it has been administered there has been only one reported death. In Sweden where it has been administered to over 22,000 patients, there have been three reported deaths.* Its value has clearly been demonstrated in breaking the drinking cycle and dependence upon alcoholic beverages "as a way of life" and in affording physicians and specialists an opportunity to rehabilitate patients physically and socially. (One of the most dramatic instances of the effectiveness of this drug involved a patient who had been admitted to Bellevue Hospital in New York 121 times in three years; since Antabuse therapy was initiated 14 months ago he has been continually "dry.")

At the Alcoholics Treatment Center in New York approximately 300 patients have been treated with Antabuse over the past three years. The experience gained with the first 149 patients during the first year's work helped us, and it may help you, in arriving at a better understanding of the drug.

Our procedure involved a thorough physical work-up, in addition to laboratory tests,† chest X-ray,† EKG† and psychometric tests. If the findings were within normal limits, the patient was given 2 gms. the first day, 1.5 gms. the second day, and 1 gm. daily on the third and fourth days. On the fourth day the patient was hospitalized and given an alcohol test dose (to be discussed later) of 1 to 3 ounces of whiskey. With these doses, even in the pre-test phase, marked toxic reactions (to be discussed later) were noted; during the test itself, the reactions were often alarming, in several cases leading to unconsciousness and collapse.

*Unpublished reports from private practitioners in Europe indicate a much higher death rate.

†Done primarily for study purposes and not believed to be essential in Antabuse therapy by the private practitioner unless indicated by the clinical findings.

Because of these reactions the dosage of Antabuse was reduced in the remaining 50 per cent of our cases. One gm. per day for the first three days was generally followed by 0.5 gm. per day for the ensuing 11 days, after which an alcohol test dose of ¾ of an ounce of whiskey was given. Following the initial test or subsequent tests, which may be required as in aversion treatment, a maintenance dose of 1.25 to .25 gm. daily was administered. Some patients have been on such doses for as long as 2½ years without any demonstrable ill effects. Habit formation in a biochemical sense, apparently does not take place.

The sensitization effect of Antabuse takes place 3–4 hours after the first dose is administered but does not reach its maximum until 12–24 hours later. According to Fox "it should *never be used for sobering up and should never be given without the patient's full knowledge and consent. Otherwise death could occur from the rapid drinking of 2–3 ounces of whiskey.*" One does not have to wait until a full week of sobriety has passed before administering Antabuse. Providing the patient is in reasonably good health and some member of his family or a friend will assume the initial responsibility of administering the drug, therapy can be initiated after one day of sobriety.

At the Alcoholics Treatment Center we have found it psychologically important to the patient—as a means of additionally impressing him with the significance of the treatment and the dangers of transgression—and medically sound to issue an identification card similar to those sometimes carried by diabetics. His card reads as follows:

> TO WHOM IT MAY CONCERN:
>
> The bearer of this card,
> (Name of Patient)
>
> is taking the drug ANTABUSE. This drug alone is harmless but, when combined with alcohol, symptoms of flushing, perspiration, redness of eyeballs, difficulty in breathing, palpitations, vomiting and low blood pressure will be observed. If such a reaction occurs and is severe, please call Dr.———— at ————————. If another physician treats patient, we suggest 1000 cc saline solution intravenously with 2 cc coramine intramuscularly. Oxygen may be necessary.

How TETD Works

Considerable work has been done here and abroad on the biochemistry and pharmacology of TETD. While all the final answers are by no means in yet, there is strong presumptive evidence in a recent study by Newman and Petzold, based on work with experimental animals and a review of earlier investigations, that TETD does not accelerate the rate of metabolism of alcohol; it may, in fact, retard it. It does, however, slow the rate of metabolism of acetaldehyde, an intermediary metabolic by-product of ethyl alcohol. This retardation in acetaldehyde metabolism with consequent build-up in the blood is believed by some investigators to be responsible for the clinical effect of TETD.

In a recent communication Christensen verified the sympathomimetic action of acetaldehyde. From his work with dogs he concluded that "the action of TETD on acetaldehyde or 1-epinephrine was to prolong the vasodilating phase, with a pronounced fall in blood pressure, an observation which explains the analogous clinical symptoms described in humans showing toxic reactions to TETD and alcohol." The intravenous injection of iron salts has been found to reverse the frequent and sometimes dramatic fall in blood pressure in the TETD-alcoholic reaction even though there is no apparent change in the acetaldehyde blood level. This action is believed to come about as a consequence of TETD combining with the iron salts, thus eliminating its action on the specific vasodilating receptor cells of the sympathetic nervous system without affecting its retardation action on the metabolism of acetaldehyde.

Indications for TETD Therapy

The following are considered indications for Antabuse therapy:

1. Good somatic health;
2. Recognition by the patient of the reality of his alcoholism and of his need for medical (and especially psychiatric) help;
3. Recognition by the patient that his alcoholic history constitutes a threat to his personal, physical and psychological well-being and, contrariwise, that TETD and psychotherapy do not constitute such a threat;

4. History of good pre-alcoholic interpersonal and sustained vocational relationships;
5. A capacity on the part of the patient to establish and utilize dependency in a therapeutic relationship.

Contraindications for TETD Therapy

Based upon the experience of a number of investigators who have been using TETD over the past three to four years, the following conditions may be considered as tentative contraindications to the use of TETD:

Medical

1. Serious cardiovascular disease;
2. Long term, serious liver dysfunction;
3. Pulmonary tuberculosis;
4. Active upper gastrointestinal disease.

Psychiatric and Social

1. History of psychotic episodes, incipient or major psychosis;
2. Subnormal or feebleminded behavior;
3. Character neuroses, long-standing and severe schizoid traits;
4. Failure to accept the reality of alcoholism and the need for medical help, with the establishment of protective barriers to help and the development of secondary anxiety reactions indicating ambivalence to treatment;
5. Long-term, inadequate interpersonal and sustained vocational relationships;

Toxic Reactions to TETD

Before sufficient clinical experience had been accumulated and dosages standardized, many patients were "normally" overdosed. Some patients to this day overdose themselves, either accidentally or because of the mistaken notion that more of a drug necessarily results in more efficacious and better actions. Generally as a consequence of overdosage but also because of individual reactions to the drug, a variety of symptoms have been observed. These may contraindicate further Antabuse therapy or may, unless relieved, so discourage the patient that he may discontinue treatment himself.

Most of the toxic effects* can be relieved by reducing the dosage or by symptomatic treatment. Among the subjective symptoms are headache, drowsiness, lethargy or "lightheadedness." Among the observable somatic symptoms are gastrointestinal upset (pain, diarrhoea, constipation), psychomotor incoordination—diplopia, speech disturbances—disassociated states and psychotic reactions. Since many of these symptoms are also commonly manifested in patients in the post-alcoholic state not on Antabuse therapy, many physicians conclude that they are essentially withdrawal symptoms. This is not the case with patients on Antabuse; their symptoms are believed to be due to the drug.

Psychotic Reactions to TETD Treatment

Psychotic reactions to Antabuse, though relatively few in number and generally transient in nature, require special discussion. In a series of 42 patients studied and treated with TETD at Blue Hills Clinic, one of the several clinics of the Connecticut Commission on Alcoholism, 8 (19 percent) developed psychotic episodes. In 5 of these, symptoms were transitory, responding promptly to the withdrawal of the drug and mild sedation and in 3 they were relatively prolonged. "Indications of mounting anxiety," according to the authors, "had been observable during the earlier TETD-alcohol reaction tests. This anxiety, together with parallel signs, can be used in helping to foresee and to handle complications that may arise in the future use of TETD."

The experience of the Blue Hills Clinic is common to others employing TETD. Martensen-Larsen, who pioneered the use of this drug, has repeatedly advised against over-dosage on the grounds that it might precipitate or produce psychosis.

The experience at the Alcoholics Treatment Center in New York has been more sanguine. In the series of 300 patients, 15 (5 percent) developed psychotic episodes, all but three of them being transitory and only one requiring institutionalization. The impression of the therapist of this one case, moreover, indicates that he probably would have required institutionalization

*Because of the danger of toxic effects, cardiac failure (particularly during the alcohol-TETD test) and other complications, the National Research Council's Committee on Problems of Alcohol has just requested the AMA's Council on Pharmacy to issue a status report for the guidance of physicians.

whether he had been treated with TETD or not. The extent to which TETD precipitated or hastened his full-blown psychosis is still conjectural but it does hold some lessons for us.

TETD—Alcohol Test Reactions

Following the oral administration of Antabuse over a period varying from 7 days to three weeks and the build-up of the acetaldehyde blood level, the patient is ready for the TETD-alcohol test. The purpose of this test is to demonstrate forcefully and dramatically that Antabuse and alcohol are violently antagonistic, that so long as the patient takes Antabuse he will be unable to drink alcoholic beverages. The test can be administered in the physician's private office if he has available a separate rest, recovery or emergency room, oxygen tank and supplies and equipment necessary to cope with conditions such as surgical shock which a violent TETD-alcohol reaction occasionally resembles. Otherwise, the test should be run in a hospital.

Before administering the alcohol test dose, the doctor will do well to recall to the patient his earlier instructions given when Antabuse therapy was first initiated, namely that Antabuse by and of itself taken in proper dosage under the physician's supervision is a harmless drug; that it is a dangerous and sometimes deadly drug if taken to excess or if drinking is initiated during the course of treatment.

Many patients having heard reports of the violence of the TETD-alcohol test reaction—some justified, some exaggerated—either refuse to take the test or become very apprehensive. Our experience indicates that the former category of patient need not be pressured into the test but can be taught the efficacy of Antabuse vicariously, by observing another patient actually undergoing the reactions. Some of these patients, who, judging by subsequent drinking "slips," their sporadic use of Antabuse and their drinking patterns, have obviously not been convinced vicariously, voluntarily return at a later date and request that the actual test be administered.

For the apprehensive, tense patient as well as the test-observer, the physician does well to explain the characteristics of possible reactions that follow the ingestion of alcohol to allay his fears and to impress him with the consequences of drinking, if he does so on the "outside."

Following these instructions the patient is generally given ¾ of an ounce to 1 ounce of whiskey. In the event that he has an extremely mild reaction or none, another ounce is administered in 30 minutes. Failure to elicit a reaction at that time should be followed by the administration of another ounce in 30 minutes. It is unusual that a reaction does not then take place.

After the alcohol test dose has been taken, the patient should be directed to lie down. A receptacle should be within easy reach should vomiting take place. An oxygen tank should be readily accessible in the event that it is needed, as should syringes with caffeine, coramine, benzedrine, epinephrine and other cardiac and/or vasoconstrictor stimulants. Blankets for warmth and bed blocks to be placed under the foot legs to accelerate blood flow back to the heart, should be available if needed.

Subjective and observable clinical manifestations within 5 to 10 minutes following the ingestion of the alcohol test dose are: progressive flushing of the face, neck and chest (it may proceed throughout the body with hands and feet "throbbing" and painful); the eyes become congested; there is a transitory rise in blood pressure and pulse rate and tachycardia. Within 10 to 20 minutes the patient has a general sense of malaise; headache may range from mild to severe; there may be respiratory difficulty and a sense of apprehension. Within 30 to 40 minutes there may be pallor, vertigo, a mild to dangerous drop in blood pressure, nausea and vomiting.

It may be generally stated that aside from individual differences the severity of these reactions depends on previous TETD dosage and the amount of alcohol administered during the test. Close clinical observation and supervision during the course of the test is therefore essential. Accordingly, lay therapists working with alcoholics should no more be permitted to administer this test than the use of other drugs.

Following one or more TETD-alcohol reaction tests, the patient may, so far as we know, be maintained on Antabuse indefinitely as has already been indicated. Eventually, through other forms of therapy, the measure of his rehabilitation and ultimate recovery can be judged not only by his freedom from dependence upon alcoholic beverages and his interpersonal ad-

justments, but by his freedom from dependence on the psychological and chemical crutch, Antabuse itself.

By itself Antabuse cannot and should not be considered a primary or sole therapeutic agent in the treatment of alcoholism. It can be considered as an effective agent in the hands of the general practitioner in making possible effective treatment by other means. Until greater interpersonal and social adjustments are achieved, however, like atabrine taken in malarial areas, Antabuse should be encouraged prophylactically especially prior to and during conventions, holidays, parties and anniversaries.

Nutrition and Alcoholism

In their studies on individual metabolic patterns, Williams and associates have proposed a genetotrophic concept of disease which, in experimental animals and in limited clinical work with human subjects, indicates that nutritional imbalance and inadequacy may have a direct bearing on the etiology of alcoholism. In animal experiments, moreover, they have found "that preventing deficiencies prevented alcohol consumption." The application of these experimental findings to humans awaits further examination before judgment can be made. There is no gainsaying the fact, however, that this line of inquiry may prove valuable, not only in the prevention, in the dependence upon alcoholic beverages, but also in the treatment of alcoholism.

On the basis of Williams' work, multivitamin capsules have been developed by Eli Lilly and Company for use in clinical trials by private practitioners.

Psychosocial Therapy in General Practice

Psychotherapy in general practice represents both an opportunity and a responsibility. In its latter aspect the difficulty in making psychiatric diagnoses, the seriousness of personality deviants among alcoholics and the danger of exacerbating the underlying condition of symptomatic alcoholics by topical and situational treatment must always be borne in mind.

Indications in deciding upon psychiatric referral for alcoholic patients are previous "nervous breakdown," history of typical psychopathy, well-defined mental illness—severe anxiety state,

hysterical reaction, delusions, hallucinations—epileptic features, and abnormal electroencephalogram, to mention a few.

If specialist psychotherapy does not seem to be indicated, most physicians are uniquely qualified in their traditional doctor-patient role to treat the more obvious and chronic psychosocial problems of their alcoholic patients.

Psychotherapy in the treatment of alcoholism in general practice, primarily of the secondary addict, is essentially a problem in human relations. It is directed only partly at the maintenance of abstinence. In the process of achieving and maintaining a goal of sobriety, the patient must be taught to understand that one drink for him is *the* one too many.

Equally vital in the therapeutic process is the joint examination by the physician and patient of the patient's personal capacities—intellectual, emotional, physical, and social—as well as his interpersonal relationships in the home, on the job, in the community. The patient must be taught to recognize, understand, and live within his capacities and limitations, not to resist them or fight them, not to reject them or attempt to escape from them via the neck of the bottle. Therapy at this level involves three R's— reassurance, reeducation, and often redirection.

The practitioner can profitably engage in substitutive treatment aimed at developing new pursuits and activities for former ones. While such treatment rarely touches underlying personality conflicts, it does afford opportunities for new and successful orientations on the part of the patient in environments less hostile and inimical than his previous ones. These opportunities are as much a part of the therapeutic process as an Rx and are often successful antidotes for C_2H_5OH.

Substitutive treatment is more than the creation of new interests and activities *per se*. It is directed at the development of new and meaningful emotional experiences from these new interests. If alcohol has meaningful content, either positive or negative, for the alcoholic, withdrawal leaves a void in his life. Here then lies a therapeutic opportunity—substituting religious, Alcoholics Anonymous, hobby, or other satisfying emotional and intellectual experiences. Discovering the precise and applicable

emotional appeals—which vary from patient to patient—is one of the fundamental challenges facing the practitioner.

* * * *

In summation, then, we can state the role of the practitioner in relationship to the alcoholic as follows: There are no magic drugs, there is no magic cure for alcoholism. Both doctor and patient must constantly bear these facts in mind. Treatment of the alcoholic calls for the best in the way of patience, persistence, fortitude and the realization that it will require months and often years of active participation on the part of both physician and patient in the therapeutic process. But such investment has already proven to be immeasurably rewarding to hundreds of physicians and thousands of patients.

BIBLIOGRAPHY

Christensen, J. A., Mechanism of the Action of Tetraethlythiuram Disulfide in Alcoholism: Iron as Antidote for the TETD-Alcohol Reaction. *Quart. J. Stud. Alc.* 12: 30–39, 1951.

Fox, R., New Techniques in the Treatment of Alcoholism, Section in Specialized Techniques in Psychotherapy, edited by Bychowski, G. and Despert, J. L., to be published by Basic Books Publishing Co. 1952.

Gottesfeld, B. H., Lasser, L. M., Conway, E. J. and Mann, H. M., Psychiatric Implications of the Treatment of Alcoholism with Tetraethylthiuram Disulfide. A Preliminary Report. *Quart. J. Stud. Alc.* 12: 184–205, 1951.

Jelliffe, Norman: Vitamin deficiencies in chronic alcoholism. In Jellinek, E. M. (Ed.): Effects of Alcohol on the Individual: Alcohol Addiction and Chronic Alcoholism. New Haven, Yale University Press, 1942, 173–262 pp.

Martensen-Larsen, O., Psychotic Phenomena Provoked by Tetraethylthiuram Disulfide. *Quart. J. Stud. Alc.* 12: 206–216, 1951.

Newman, H. W. and Petzold, H. V., The Effect of Tetraethylthiuram Disulfide on the Metabolism of Ethyl Alcohol. *Quart. J. Stud. Alc.* 12: 40-45, 1951.

Smith, J. J.: A Medical Approach to Problem Drinking: Preliminary Report. *Quart. J. Stud. Alc.* 10: 251–257, 1949.

Voegtlin, W. L.: Limitations and Adjunctive Therapies in Treatment of Chronic Alcoholism. *M. World* 65: 165–168, 1947.

Williams, R. J.: Nutrition and Alcoholism. Norman, Oklahoma: University of Oklahoma Press. 1951. pp. x plus 82. Individual Metabolic Patterns and Human Disease: An Exploratory Study Utilizing Predominantly Paper Chromatographic Methods. Biochemical Institute Studies IV. University of Texas Publication, Pbl. 5109, May 1, 1951.

THE POSSIBLE ROLE OF THE COCKROACH IN THE DISSEMINATION OF POLIOMYELITIS VIRUS*

CHIN MIN HSIANG, MORRIS POLLARD, AND D. W. MICKS

The incrimination of the cockroach in the dissemination of some virus diseases has been an interesting problem in the fields of virology and entomology. Syverton and Fisher (1950) fed cockroaches *(Periplaneta americana)* a single meal containing the virus of mouse encephalomyelitis, GD VII strain, and they found that the test cockroaches daily defecated sufficient virus to kill test mice over a period of as many as 7 days. Fisher and Syverton (1951) also found that the same strain of cockroaches, fed a single meal containing a Coxsackie virus, were able to defecate, daily, sufficient virus to paralyze and kill the test mice over a period of 15 days.

Whether the cockroach plays a role in transmitting poliomyelitis viruses still remains equivocal. Hurlbut (1951) found that 13 days after the injection of Lansing poliomyelitis virus into the hemocoele of the cockroach *(Periplaneta americana)* it was possible to demonstrate virus by triturating the whole inoculated cockroach and passing it to normal mice. In spite of this, the natural role of cockroaches in the epidemiology of poliomyelitis is still rather inconclusive. The present communication makes known some findings that were obtained experimentally when the American cockroach *(Periplaneta americana)* was tested for its ability to serve as an enteric carrier of the Lansing poliomyelitis virus.

Materials and Methods

Virus: The Lansing poliomyelitis virus, in the form of a 20% cotton rat brain emulsion in sterile normal saline with titer LD_{50} 10^{-4}, was used in all experiments.

Cockroaches: The cockroaches *(Periplaneta americana)* were collected in a grain elevator basement in Galveston, Texas, far

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removed from human habitation. Adults as well as mature nymphs were used.

Roach mounting and virus feeding: After trying unsatisfactorily the method of mounting cockroaches as described by Wedberg *et al.* (1949), a more simple and convenient method was devised. Roaches, anesthetized with CO_2 (1950) were attached on their dorsal surface to paraffin in a tray as shown in Figure I. The roaches were left in the tray overnight without food and water in order to stimulate their appetite. Fifty or 60

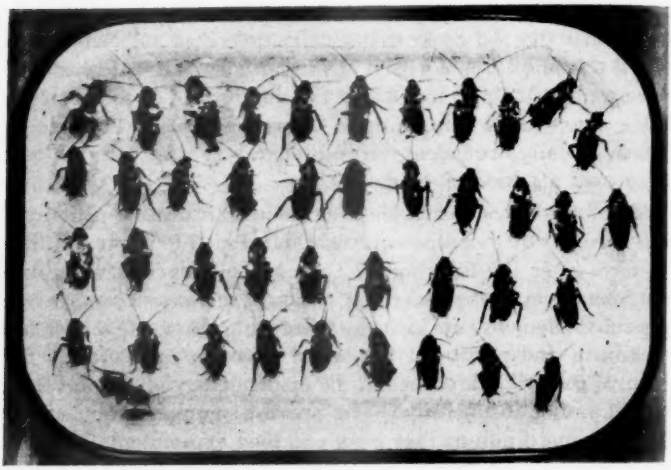

FIG. I. Roaches mounted on paraffin.

roaches were mounted in one tray and each roach quite readily consumed 0.1 to 0.2 cc. of the virus suspension (average dose about 2000 cotton rat LD_{50}) within two minutes. A 1.0 cc. tuberculin syringe with a 25 gauge needle was used to perform the feeding. After a single meal given this way, the roaches were then released into a wire cage (Fig. II) where they could take water and food more freely. The fecal specimens were collected in a petri dish at the bottom of the cage at 24-hour intervals. All specimens were stored at $-20°$ C. until preparation for mouse inoculation. The experiments were repeated three times.

Inoculum preparation and mice inoculation: Feces or tissues of

the cockroaches were prepared in 20% suspensions with sterile normal saline and centrifuged at 5000 RPM for thirty minutes. Then, 1500 units of penicillin and 500 gamma of streptomycin were added to the supernatant. The latter was then centrifuged again at 18,000 RPM for fifteen minutes. The supernatant was then inoculated into 6 white Swiss mice (0.03 cc. intracerebrally) and each mouse was observed for the following 21 days.

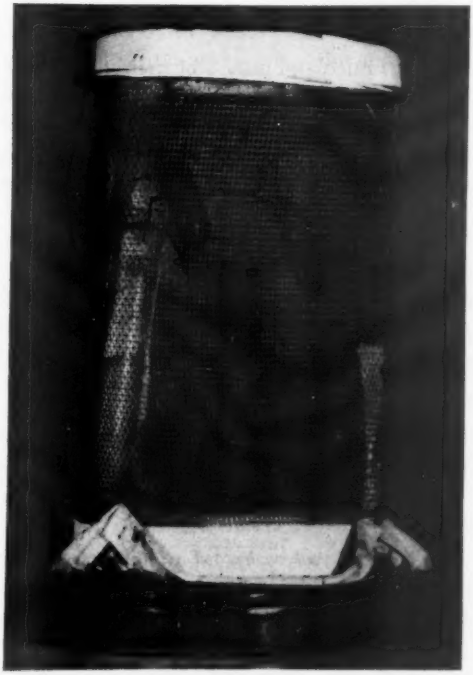

FIG. II. Cage for roaches after virus feeding. Feces collected in the petri dish at the bottom of the cage.

Two of 6 mice inoculated with the feces collected within 24 hours (Table 1) after the virus meal showed paralysis and death at the 9th and at the 20th day after inoculation. (The brain of the second paralyzed mouse was emulsified and inoculated into

Results of Experiments

TABLE 1

Test for virus in feces of cockroaches after a single meal of Lansing virus.

Specimens collected days after virus meal									
1	2	3	4	5	6	7	8	9	10
2/6*	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6

*Number paralyzed/number of mice inoculated.

another group of 5 mice and only 1 of them developed paralysis 3 days after inoculation.) These data indicate that a small quantity of paralyzing virus appeared in the feces of cockroaches on the first day after a meal containing the virus. No virus could be detected in the fecal specimens collected for the subsequent 9 days.

Since virus was recovered from roach feces within only 1 day after the virus meal, it was of interest to see whether the virus still existed within the roach. Sixty roaches, fed with a virus meal, were dissected in groups of 10 at daily intervals after virus feeding. (Some of the roaches died before dissection and were discarded.) The heads and intestines were pooled separately and inoculated into mice.

The results (Table 2) indicate that no virus could be detected in roach tissues even within 24 hours after the virus meal.

TABLE 2

Test for virus in tissue of cockroaches fed a single meal of Lansing virus.

Specimens collected days after virus meal									
1		2		3		4		5	
head	int.	head	int.	head	int.	head	int.	head	int.
0/6*	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6

*Number paralyzed/number of mice inoculated.

Since virus neither survived inside the roach tissues nor could be detected in a reasonable quantity in their feces, the question arose whether the roaches' tissue or its feces may be toxic to the

Lansing virus which was used in this experiment. Therefore, the following experiment was carried out:

Pools of different parts of 10 cockroaches (wings and legs removed), two whole roaches and normal roach feces were ground separately to make up 10% suspensions in normal saline and processed as has been described above. These suspensions, after proving non-toxic to the test mice, were mixed with Lansing virus very carefully in the ratio of 10 to 1. These mixtures were incubated in a cool room (25°C) for ninety minutes or in a refrigerator (4°C) for 6 days before inoculation into mice. Virus mixed with normal saline was used as the control.

Table 3 shows that neither tissue suspensions nor feces of the cockroach has the ability to destroy the Lansing virus over a period of 6 days *in vitro* at low temperature.

TABLE 3

Test for virus in mixtures of roach tissues plus virus

Virus mixed with					
feces	head	intestine	body	whole roach	saline
25°C 90 minutes					
3/3*	3/3	3/3	3/3	3/3	3/3
4°C 6 days					
3/3	3/3	3/3	3/3	3/3	3/3

*Number paralyzed/number of mice inoculated.

Discussion

The American cockroach *(Periplaneta americana)* may contribute to the dissemination of poliomyelitis virus from infected excreta, but under the conditions of our experiments this could not be demonstrated. Cockroaches fed with Lansing poliomyelitis virus defecated small amounts of virus for only one day. Two explanations were considered: 1. Either the virus is passed rapidly through the roach without propagation therein; or 2. The virus is inactivated through roach tissue metabolism. While the agent thus isolated from the roach feces induced poliomyelitis-like syndromes in the inoculated mice, it was not identified by serological means. Such an effect may not have been due to

poliomyelitis virus; however, no such syndrome could be induced with feces collected from normal roaches. Rendtorff and Francis (1943) found that Lansing virus remained in the gut of flies, *Musca domestica* for no longer than 49 hours and they attribute this to rapid excretion of the virus. This may very well occur in the roach. Since roach tissues were not destructive or neutralizing to the virus, *in vitro*, this suggests the possibility that metabolizing roach tissues *in vivo* exert the destructive effect described above. The possibility that this or other species of cockroaches in the *normal* process of feeding on excreta from human sources may acquire virus for later transfer by contamination of food is still open to speculation.

The dry character of roach feces may adversely influence the survival of virus after defecation. Perhaps under conditions of higher environmental humidity, the virus inactivating effect of drying might have been averted. On the other hand, the virus could not be recovered directly from the intestinal content of virus-fed roaches. Faber *et al.* (1951) and Pollard (1951) reported that desiccation exerts a prompt and marked inactivating effect on poliomyelitis virus and that even a small amount of residual moisture might favor continuing activity. One would not expect roach feces to attain complete desiccation in this laboratory, since the average relative humidity in Galveston during the period of study was 62 low and 92 high.

Summary

When cockroaches *(Periplaneta americana)* were fed a single meal containing Lansing poliomyelitis virus, they defecated detectable virus only for one day after the virus meal. No virus inactivating effect of normal roach tissues or of normal roach feces could be demonstrated *in vitro*. Since the intestinal content of fed roaches contained no virus within 24 hours after feeding, this suggests that virus inactivation might result either from metabolic effect or from rapid excretion of the virus from the gut of the roach.

REFERENCES

Faber, H. K., Dong, L., and Silverberg, R. J., A Search for Poliomyelitis Virus in House Dust and a Study of the Effects of Drying on the Viability of the Virus, *J. Inf. Dis.*, 88:180, 1951.

Fisher, R. G. and Syverton, J. T., The Cochroach as an Experimental Vector of Coxsackie Virus, *Am. J. Trop. Med.*, 31:238, 1951.

Hurlbut, H. S., The Recovery of Poliomyelitis Virus After Parenteral Introduction into Cochroaches and Houseflies, *J. Inf. Dis.*, 86:103, 1951.

Olson, T. A. and Rueger, M. E., Experimental Transmission of *Salmonella Oranienburg* Through Cockroaches, *Public Health Reports*, 65:531, 1950.

Pollard, M., The Inactivation of Poliomyelitis Virus by Freeze-Drying, *Texas Rep. Biol. & Med.*, 4:749, 1951.

Rendtorff, R. C. and Francis, Thomas, Jr., Survival of the Lansing Strain of Poliomyelitis Virus in the Common House Fly, *Musca Domestica*, *J. Inf. Dis.*, 73:198, 1943.

Syverton, J. T., and Fisher, R. G., The Cockroach as an Experimental Vector of the Virus of Spontaneous Mouse Encephalomyelitis (Theiler), *Proc. Soc. Exp. Biol. and Med.* 74:296.

Wedberg, *et al.*, The Passage of Microorganisms Through the Digestive Tract of *Blaberus Cranifer* Mounted Under Controlled Conditions, *J. Bact,*. 58:573, 1949.

TISSUE CULTURE STUDIES ON HUMAN SKIN

III. Some Cytological Features of the Outgrowth of Epithelial Cells*

T. C. Hsu†

In vitro studies on human skin have gained increasing attention in recent years. Several reports from this laboratory have been published dealing with a short-term culture technique for epidermis in hanging drop preparations. The morphological features of epithelial cells studied with phase contrast microscopy (Lewis, Pomerat and Ezell, 1949), culture methods and nutritional improvements for tissue outgrowth (Ulloa-Gregori, Blocker, Nowinski and Pomerat, 1950), drug toxicity (Everett, Pomerat, Hu and Livingood, 1951), the effect of allergens (Everett, Livingood, Pomerat and Hu (in press)), and radioactive substances (Hu, Holmes, Pomerat, Livingood and McConnell, 1951) have been described. Methods of culture and the estimation of the amount of outgrowth are gradually becoming standardized.

The present study deals with the outgrowth and proliferation in adult human epidermis with special reference to the behavior of the cells during mitosis.

Materials and Methods

Remnants from skin grafting procedures were obtained with an electric dermatome through the courtesy of the Department of Plastic Surgery. These were cut into squares approximately 2 mm. on each side with sharp razor blades. Hanging drop cultures were made in the usual way (cf. Ulloa-Gregori, Blocker, Nowinski and Pomerat, 1950; Everett, Pomerat, Hu and Livingood, 1951), i.e., utilizing one drop of chick embryonic extract (embryonic juice diluted with an equal volume of Gey's balanced salt solution) and one drop of rooster plasma

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to form the clot, and four drops of either human serum or malignant ascitic fluid as the fluid nutrient. Penicillin was added in the plasma to make a final concentration of 1,000 γ per ml. of medium.

The cultures were incubated at 37°C and fixed with methyl alcohol after various periods of incubation, from two to thirteen days. With the exception of a few cultures tested with Feulgen's reagent all were stained according to Jacobson's method (Orr, Hardy and Pomerat, 1950).

Grateful acknowledgment is made to Doctors C. M. Pomerat, Ulloa-Gregori, Funan Hu and Mr. George Crosby for advice and indispensable technical assistance.

Results

I. *Cell size*

It had been noticed in skin cultures that cells enlarged considerably during the course of their emigration. Any attempt to measure the extent of increase in volume encounters difficulty since the epithelial cells at the margin of the outgrowth are very irregular in shape due to membrane activity and to spreading of the cytoplasm with consequent lack of uniformity in cell thickness. An indirect and admittedly inaccurate method of measurement is possible, however, which serves to indicate the extent of increase in cell area without regard to volume. By adjusting the camera lucida projection to a level where three millimeters on graph paper represented one micron, a standard weight relation curve was obtained as follows: At a scale of 1 μ to 3 mm., paper cut-outs of sizes representing 100 μ^2, 400 μ^2, 900 μ^2, 1,600 μ^2, 2,500 μ,^{2} and 3,600 μ^2 were weighed, each value being the average of ten pieces of paper. The resulting graph showed a satisfactory straight line relationship (fig. 1). With the camera lucida projection adjusted to this same scale of 1 μ to 3 mm., cells from the edge of the outgrowth were sampled and the outline of each was drawn on the same brand of bond paper as was used in constructing the standard curve (American Trust Bond). Each outline was then cut out and weighed. The weight of the paper representing any one cell could thus be converted to cell area by consulting the standard curve.

This method of measurement contains several possible sources of error. First, there is an inherent sampling error. The cells at the margin of outgrowth have different degrees of membrane activity and hence vary greatly in size. However, caution was

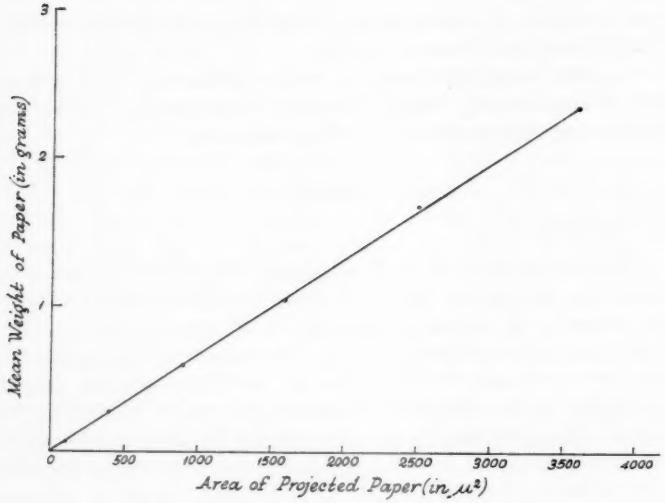


FIG. 1. Standard curve for the weight-area relation of the paper used to evaluate cell and nuclear areas.

taken to avoid bias by random sampling in such a way that cells at the margin of the outgrowth were drawn without choice, unless they overlapped or the cell margins were not clearly marked. Secondly, in the processes of drawing, trimming, and weighing, slight errors might be introduced but with due care it was felt that the errors of sampling, drawing and weighing were so slight as to be insignificant.

The weights of the individual cells projected on the paper were recorded separately and the variance of each cell population calculated. Four sets of cultures were made for this purpose, two grown in human serum and two in ascitic fluid. The

results are shown in Table I. The last column of Table I and the curve in figure 2 represent the combined values of all the 1,179 cells measured, which is probably more accurate because

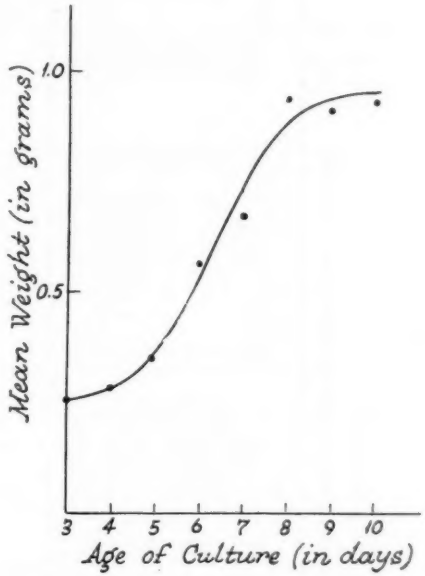


FIG. 2. Mean weight of paper outlines of projected cells.

of the larger sample. Thus the mean weight of the paper patterns of the three-day cells was 0.259 grams, representing an approximate area of 380 μ^2, whereas the four-day cells had a mean weight of 0.283 grams, representing approximately an area of 420 μ^2. The increase in cell size became more rapid after four days and reached an area of approximately 1,400 μ^2 at eight days, which is about 3.68 times as large as the three-day cells. The largest cell so far recorded exceeded 4,500 μ^2 and cells over 2,000 μ^2 in size were not uncommon. After eight days the cells no longer increased in mean size. The curve

from three to ten days is somewhat sigmoid in shape, indicating that the increase in the earlier and later days is slight

TABLE I

Mean weight and variance (in grams) of paper outlines of projected cells

Days of cultures	Samples 1 Mean weight	1 No. cells	2 Mean weight	2 No. cells	3 Mean weight	3 No. cells	4 Mean weight	4 No. cells	Total Mean weight	Total No. cells
3	.....	...	0.268±0.005	64	0.224±0.003	86	0.323±0.006	40	0.259	190
4	0.303±0.006	132	0.248±0.004	26	0.258±0.008	69	.....	..	0.283	227
5	0.409±0.014	112	.....	..	0.442±0.028	31	0.485±0.023	67	0.449	210
6	0.595±0.034	34	0.468±0.015	30	0.581±0.061	40	0.599±0.027	49	0.568	153
7	0.551±0.018	37	0.715±0.033	22	0.649±0.049	49	0.768±0.074	34	0.664	142
8	0.768±0.011	29	0.790±0.042	20	0.912±0.092	36	1.119±0.275	44	0.936	129
9	.....	..	.....	..	.....	..	0.910±0.065	59	0.910	59
10	.....	..	.....	..	0.792±0.026	35	1.070±0.125	34	0.929	69

but is rapid in the middle period of from four to eight days. Presumably the cells at two days could not be much smaller than those of three days but after 48 hours of incubation most of the cultures showed only a few migrating cells.

II. *Nuclear size*

A method similar to that employed for cell size was used for measuring nuclear size. The data presented in Table II are

TABLE II

Mean weight and variance (in grams) of paper outlines of projected nuclei

Days of cultures	Mean weight	Variance	Number of cells
3	0.051	0.0002	175
4	0.052	0.0005	212
5	0.060	0.0005	179
6	0.078	0.0005	184
7	0.095	0.0010	217
8	0.089	0.0004	174
9	0.094	0.0006	158
10	0.106	0.0010	126

the combined figures for all 1,425 nuclei measured. The curve of increase in nuclear size (fig. 3) is not as smooth as the one

FIG. 3. Mean weight of paper outlines of projected nuclei.

for the cell size. When the nuclear sizes (weights of paper) were grouped per 10 milligrams of increase (Table III and fig. 4), it could readily be seen that on the seventh and the

TABLE III

The distribution of weights (in grams) of paper outlines of projected nuclei

Arbitrary weight classes	Days of cultures								Total number of cells
	3	4	5	6	7	8	9	10	
0.02–	..	11	8	..	..	..	..	..	19
0.03–	38	61	27	3	..	..	..	..	129
0.04–	47	59	36	11	4	1	..	2	160
0.05–	54	22	20	14	13	7	3	3	136
0.06–	20	14	30	37	33	14	13	5	156
0.07–	10	16	26	41	26	34	31	9	193
0.08–	4	14	18	35	28	40	39	15	193
0.09–	1	5	7	24	40	41	26	25	169
0.10–	0	7	3	11	16	14	12	21	84
0.11–	1	1	0	4	21	16	11	13	67
0.12–	..	1	3	3	10	1	7	7	32
0.13–	..	0	1	0	5	1	8	13	28
0.14–	..	1	..	1	6	2	2	6	18
0.15–	..	..	..	1	3	1	2	1	8
0.16–	..	..	..	..	4	1	0	2	7
0.17–	..	..	..	..	3	1	3	1	8
0.18–	..	..	..	..	1	..	0	0	1
0.19–	..	..	..	..	2	..	1	0	3
0.20–	..	..	..	..	0	..	..	0	0
0.21–	..	..	..	..	1	..	..	1	2
0.22–	..	..	..	..	1	..	..	0	1
0.23–	..	..	..	..	..	..	..	2	2
Total number of cells ..	175	212	179	184	217	174	158	126	1,425

tenth days there were several very large nuclei which greatly affected the means and the variances. From Table III it is apparent that not only the modes of the weights are shifting towards the heavier side as the cultures grow older, but a more dispersed distribution of the weight classes is observed. Some of the nuclei in later days were as large or even larger than the cells themselves which were measured in the early days of the culture. The increase in nuclear size is obvious, but the question remains as to whether it is due to actual growth. However, mere imbibition, flattening or other physical changes probably could not account for the increment which was observed, because some of the cells at division stages showed that they were polyploids.

Polyploid cells are not uncommon in normal tissues and the three-day sample also showed the existence of low polyploid

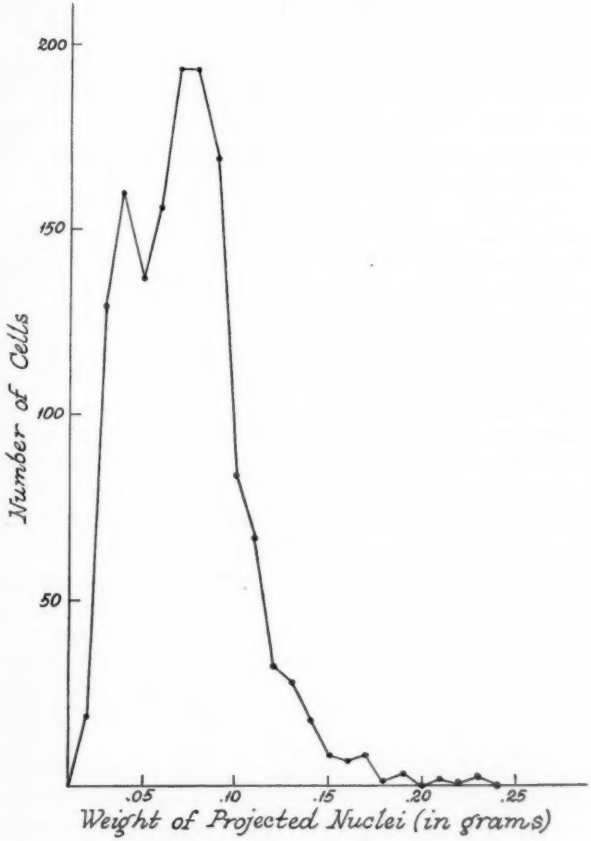


FIG. 4. Distribution of the weight (in grams) of paper outlines of projected nuclei.

cells, but since the number of the polyploid cells seemed to increase gradually and the degree of ploidy also seemed to increase, it would mean that endomitosis had been taking place

in the course of cultivation. The total frequency of the weight classes represented in figure 4 also shows a discontinuous distribution of the nuclear area with a marked drop of the frequency between 0.05–0.069 grams.

III. *Mitosis*

It has been claimed that mitotic divisions occur in epidermal cells *in vitro*. Thus, in figure 1 of the article by Blocker, Pomerat and Lewis (1950), there are three obvious mitotic figures, two in late anaphase and one in metaphase. It becomes important to know something of the extent of cell division under these conditions.

Cultures fixed at various days of incubation were examined for the presence of mitoses. A number of slides made by Dr. Ulloa-Gregori in connection with the effect of adding burned-skin extracts to cultures of skin were also included. Of the slides examined, with or without the skin extract treatment, mitosis was, by and large, a rare event. In the early stages of incubation, from two to four days, no mitotic figure had been recorded. Cells in division first occurred in the five-day cultures and, although they were more frequent in later days, they were never numerous.

It is impractical to record the number of cells in mitosis in percentages of the whole cell population since, proportionally, they are rare even in the slides containing the largest number of mitotic figures. A rougher recording was made by estimating the amount of the outgrowth and expressing this with an arbitrary plus rating as employed by Everett, Pomerat, Hu and Livingood (1951) and by recording the number of cells in the stages of division. The results of observations on 240 slides are grouped in Table IV.

Superficially, it appears as if the multiplicative phase of growth, though at an extremely low rate, does exist. However, a closer study of dividing cells reveals that it is very doubtful that these mitotic figures really indicate a consistent pattern of growth. First, it was found that only a few of the slides contained mitotic figures (32 out of 240) and most of these were in even fewer slides, with one containing 89 and another 33. The presence of such a large number in these two preparations remains unexplained. Unlike some other tissues *in vitro*

in which one can expect a certain proportion of cells in mitosis at a given age, the frequency of mitosis in skin cultures was erratic.

Secondly, most of the dividing cells probably did not complete their normal course of mitosis because degeneration occurred after the metaphase. Prophases were mostly normal and the metaphase was reached. Figures 5 and 6 show two different cells at full metaphase with clearly defined chromosomes and a well developed plate of orientation. However, very few cells evidently passed normally beyond this stage; either the chromosomes shortened and aggregated at the center of the cell (fig. 7) and finally reached a stage represented by a mass of pycnotic chromatin material (figs. 8 and 9), or they divided abnormally into various expressions of anomalous anaphase (figs. 10 and 11). A few normal anaphases have been observed in one slide which was treated with burned-skin extract and which contained 89 division figures.

When the dividing cells totalled in Table IV are grouped according to their phases of division, an extremely high proportion of metaphases is found. Out of the 215 cells, 53 are in prophase, 153 in metaphase, 8 in anaphase and one in telophase. Ordinarily, prophase takes a much longer time than other stages and hence it should be represented by more cells in a normally dividing cell population. In contrast, the metaphase dominates in this series and is strongly suggestive that the dividing cells were stopped at this point and did not continue their mitotic course. It appears that there is very little multiplicative growth in this type of skin culture.

Polyploid cells were more frequent in older cultures and occasionally some of these cells were in division stages. Figure 12 shows a high polyploid nucleus in mid-prophase and figure 13 is probably a tetraploid cell in full metaphase.

It has been observed that the cells near the edge of these skin explants usually stained weakly. Feulgen reaction demonstrated that the nuclei of these cells had a low content of desoxypentose nucleic acid. This is particularly clearly seen in the dividing cells as shown in figure 14, in which the chromosomes are slender and very faintly charged with nucleic acid.

TABLE IV

The incidence of mitosis in skin cultures

Days of cultures	Nutrient fluid	No. of cultures	Total outgrowth (in plus ratings)	No. slides with mitotic figures	No. cells in mitotic stages	Phases of mitosis
2	as.fl.	8	8.5	0	0	
3	as.fl.	12	29	0	0	
5	as.fl.	12	30.5	2	4	PM
6	as.fl.	5	16	1	3	P
7	as.fl.	27	103	6	40	PMA
8	as.fl.	23	67	6	19	PM
9	as.fl.	12	32.5	2	6	PM
10	as.fl.	8	44	0	0	
11	as.fl.	7	17	0	0	
12	as.fl.	4	9	0	0	
13	as.fl.	2	6	0	0	
3	sera	2	4	0	0	
4	sera	3	4.5	0	0	
5	sera	2	3	0	0	
6	sera	10	9.5	0	0	
7	sera	7	14	0	0	
8	sera	9	8.5	0	0	
9	sera	2	2.5	0	0	
10	sera	10	16.5	2	15	PM
6*	b.s.†	10	20	2	5	PM
6*	b.s.	10	24	1	1	M
6*	b.s.	10	24	4	31	PM
6*	b.s.	10	29	4	89	PMAT
6*	b.s.	10	23.5	1	1	M
6*	b.s.	10	32	1	1	A
6*	b.s.	10	33	0	0	
6*	b.s.	5	17	0	0	
Total		240		32	215	

Phases of mitosis: P—Prophase, M—Metaphase, A—Anaphase, T—Telophase.
*From slides made by Dr. Ulloa-Gregori.
†Burned-skin extract with ascitic fluid.

Moreover, the chromosomes of this cell appear in a C-mitosis-like fashion, with the chromatids and centromeres separated but still remaining close to each other in pairs. The cause of this type of behavior is unknown, but nucleic acid deficiency

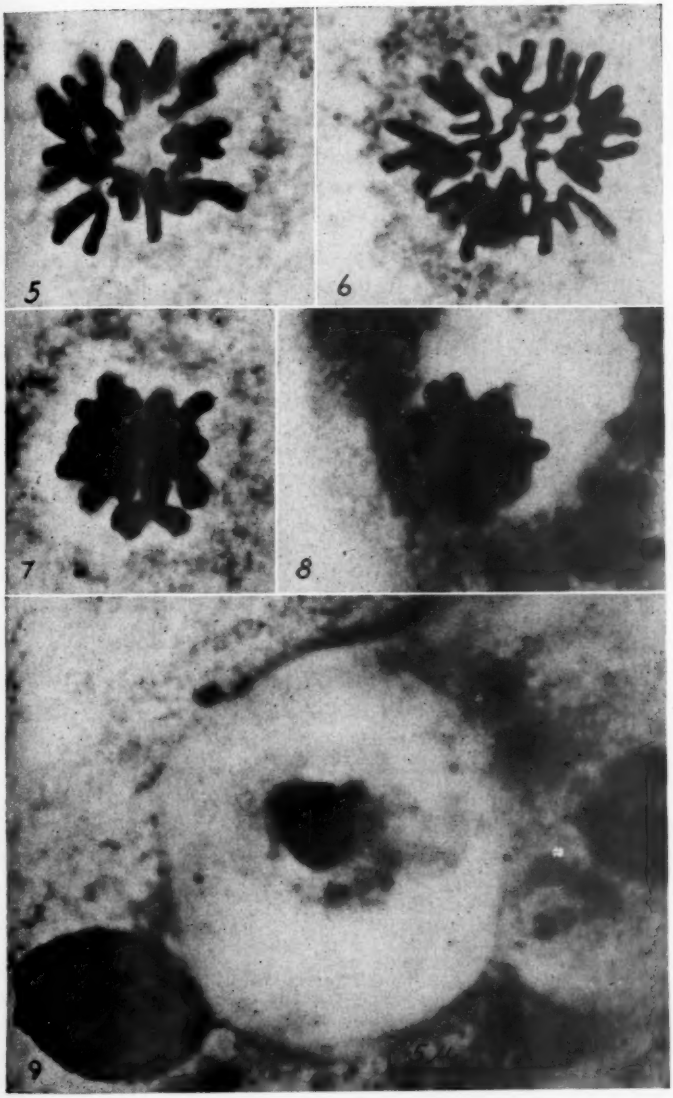
FIGS. 5–9. Mitotic figures in epidermal cells from human adult skin in simple hanging drop cultures. Magnification is indicated by the scale in figure 9. All preparations stained with Jacobson's method.

FIGS. 5 and 6. Typical normal metaphases.

FIG. 7. Early stage of degeneration showing shortening and clumping of chromosomes.

FIG. 8. Later stage of chromosome degeneration.

FIG. 9. Terminal stage in chromosome degeneration.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9

may be explained in that the cells migrate from the explant and take some nutrients from the plasma clot, making the area near the explant almost devoid of nutrients. The cells emigrating later can take nutrients only from the fluid phase and therefore have a smaller nutritive supply, becoming but poorly charged with nucleic acid.

Discussion

If growth is defined as (1) cell division or multiplication and (2) increase of volume in terms of actual manufacture of cell material, then human epidermal cells in hanging drop cultures prepared according to the method described do not appear to fulfill these conditions. True cell division is very rare. Mitoses are observed, occasionally in large numbers in some particular slides but the majority of the cells undergo degeneration after reaching metaphase. Whether these cells die thereafter or become polyploids after a reconstitution process could not be determined. At any rate, it is not considered that these division figures are a part of the growth phenomena, especially since they always appear in the later period of their life in culture. Hence the cells which are found in the outgrowth must have come mainly from the explants as the result of emigration.

Bullough and Johnson (1951) were able to maintain mitosis *in vitro* in epidermal cells from ear fragments of mice. However, their experiments lasted only a few hours and do not necessarily mean that mitotic activity could have been maintained for several days. In this laboratory, no signs of mitoses have been observed in several squash preparations of skin explants after several days in culture when stained with acetoorcein.

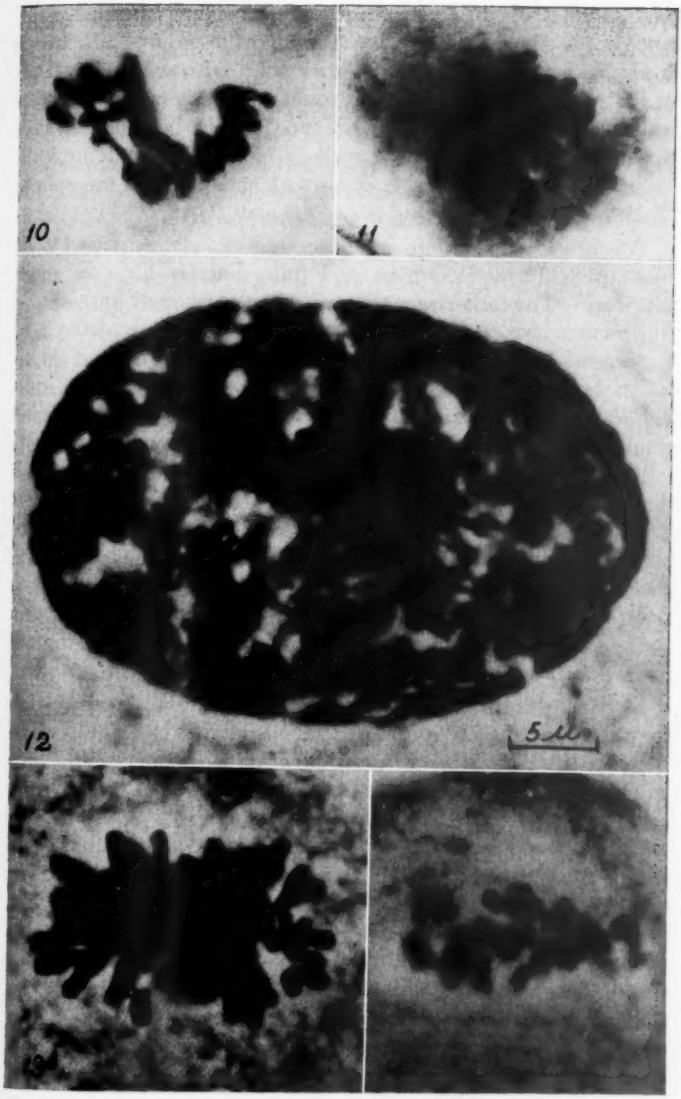
FIGS. 10–14. Mitotic figures in epidermal cells from human adult skin in simple handing drop cultures. Magnification is indicated by the scale shown in figure 12. All preparations stained with Jacobson's method.

FIGS. 10 and 11. Anomalous anaphases.

FIG. 12. Mid-prophase of a highly polyploid nucleus.

FIG. 13. Metaphase of a probable tetraploid cell.

FIG. 14. A C-mitosis-like plate with chromosomes poorly charged with nucleic acid. Note that the centromeres are separate but the chromatids show no evidence of moving to the poles.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5 μ

Another possibility which has not been fully examined is the age of the skin donor. All the samples used for this paper were from adult patients. Whether skin cultures from infants or at least of young children would show higher rate of cell multiplication as is the case *in vivo* should be studied. Preliminary work with skin from a four-month human fetus indicates that the mitotic cycle is normal and that the metaphase block noted in adult tissue is not present (Hsu, 1952).

The other phase of growth, increase of cell constituents, is more difficult to determine and the result is likewise more uncertain. The cell-size curve obtained, although resembling a growth curve, is probably not of this category inasmuch as it does not deal with increased cell numbers in a given population but only with the increase in area of the mean individual cell. Imbibition as well as the activity of the membrane might play an important part in the enlargement of the cells without an accompanying change in volume. Similarly, if no direct evidence of polyploids is obtained, the increase of nuclear area can tell little about volume because nuclei, although more rigid, also might be influenced by agencies such as flattening and hydration. However, since it is reasonably certain that polyploid nuclei arise as the cultures grow older, it is also reasonable to assume a certain degree of cellular growth though more accurate determinations are needed to clarify the situation.

In tissue culture, epidermal cells provide a good testing material for cell activity and metabolism. One of the phases of such studies makes use of cinematographic records of the cells in relation to various environmental factors. For this purpose, larger cells with the greatest activity of the cytoplasmic membrane are recommended. The hanging drop cultures as used in this study, though sometimes maintained more than ten days, usually start to degenerate after one week. Taking this into consideration with the fact that the size curve shows that the cells reach their maximal average size at eight days, it is suggested that for perfusion chamber experiments (Pomerat, 1951), cultures incubated for seven days are preferable.

As can be seen from Table IV, the majority of the dividing cells were found in experiments in which the skin cultures were treated with burned-skin extracts. Moreover, out of eight cells

in anaphase only one was recorded from a seven-day culture without treatment while the rest of them were found in the burned skin experiments of Dr. Ulloa-Gregori. However, even in these slides, metaphase degeneration was still a dominating feature. It is not very convincing that the burned-skin extract had anything to do with the cell multiplication of the skin cultures but the question should be reinvestigated.

These observations of incomplete or adnormal mitotic activity in tissue cultures of human skin seem to suggest that in a strict sense we are dealing only with "survival" cultures of emigrating cells, since there is no actual increase in the number of elements present. Cells definitely increase in size and perhaps in volume, but the cellular aggregation of the explant is a dying system where degeneration instead of growth takes place. However, for short-term experiments the usefulness of epidermal cells is not vitiated by these facts for the cells remain reasonably active and viable for approximately one week.

For long term experiments and where actual growth is desirable, a new approach to the nutritional requirements to maintain normal and complete mitosis is necessary. An effort to analyze the significant factors involved has been described by Parshley and Simms (1950).

Summary

1. In an analysis of 1,179 cells in hanging drop cultures of adult human skin, epidermal cells in the outgrowth area showed an increase in cell area at a rate that followed a sigmoid curve. The rate of increase was slow during the first four days of culture but became rapid after four days and rose sharply until the eighth day, at which time increase again became slowed.

2. In 1,425 cells measured an increase in nuclear size approximated the rate of that for the entire cell area but polyploidy became common and indicative of endomitosis.

3. Epidermal cells incubated for seven days which are large and active are recommended for perfusion chamber experiments.

4. Complete and normal mitoses are rare in these cultures, perhaps indicating cellular malnutrition. Out of 240 slides

studied only 32 contain mitotic figures. Most cells in mitosis either degenerate after metaphase or give anomalous anaphases. Work recently begun with fetal skin indicates a normal mitotic course for embryonic epidermal cells and suggests a "growth propensity" lacking in adult epidermis that permits normal mitoses.

LITERATURE CITED

Blocker, T. G., Jr., C. M. Pomerat and S. R. Lewis: Research opportunities with the use of cultures of living skin. *Plastic and Recon. Surg.*, 5:283–288, 1950.

Bullough, W. S. and M. Johnson: A simple technique for maintaining mammalian epidermal mitosis *in vitro*. *Exp. Cell Res.*, 2:445–453, 1951.

Everett, E. T., C. M. Pomerat, F. N. Hu and C. S. Livingood: Tissue Culture Studies on Human Skin. I. A method of evaluating the toxicity of certain drugs employed locally on the skin. *Texas Rep. Biol. and Med.*, 9:281–291, 1951.

Everett, E. T., C. S. Livingood, C. M. Pomerat and F. N. Hu: Tissue Culture Studies on Human Skin. II. Comparative effects of certain specific contact allergens on sensitized and non-sensitized human skin. *J. Inv. Derm.* (in press), 1952.

Hsu, T. C.: Human Chromosomes *in vitro*. I. The karyotype of man. *J. Heredity* (in press), 1952.

Hu, F. N., S. G. Holmes, C. M. Pomerat, C. S. Livingood and K. P. McConnell: A method for studying the effect of P^{32} on living adult human epidermal cells in a perfusion chamber. *Texas Rep. Biol. and Med.*, 9:739–748, 1951.

Lewis, S. R., C. M. Pomerat and D. Ezell: Human epidermal cells observed in tissue culture with phase contrast microscopy. *Anat. Rec.*, 104:487–504, 1949.

Orr, M. F., M. H. Hardy and C. M. Pomerat: W. Jacobson's method for staining whole tissue cultures differentially for ribo- and desoxyribonucleoproteins. *Anat. Rec.*, 106:143–144, 1950.

Parshley, M. S. and H. S. Simms: Cultivation of adult skin epithelial cells (chicken and human) *in vitro*. *Am. J. Anat.*, 86:163–183, 1950.

Pomerat, C. M.: Tissue Culture Methods. *Methods in Medical Research, Volume IV*. Year Book Publ., 198–291, 1951.

Ulloa-Gregori, O., T. G. Blocker, Jr., W. W. Nowinski and C. M. Pomerat: The effect of ascitic fluid and of blood serum on the outgrowth of epidermal cells from the human skin in tissue culture. *Texas Rep. Biol. and Med.*, 8:400–409, 1950.

MUSCLE TEMPERATURES IN RABBIT LEGS DURING VARIOUS CONDITIONS OF COLD EXPOSURE AND REWARMING*

ROBERT B. LEWIS AND ELLA FREYTAG

Introduction

In another investigation (Pichotka, Lewis, and Freytag, 1951) it was demonstrated that after a standard cold exposure of a rabbit's leg, muscle necrosis first became manifest in the tibialis anticus near the junction of the middle and distal thirds. The second most common sites of muscle necrosis were the distal portions of the extensor digitorum longus, flexor digitorum longus, and peronaei. The triceps surae (gastrocnemius plus soleus) was the last to show necrosis.

The purpose of this investigation was to determine the temperature course in different muscle areas of the rabbit's leg during exposure to various bath temperatures and conditions of thawing.

Materials and Methods

Continuous temperature recordings were made by means of a multipoint Brown potentiometer using copper-constantan thermocouples (30-gage wire) placed in various muscle areas of the leg. Under light pentothal sodium anesthesia the thermocouple wires were threaded transversely through the entire thickness of the leg by means of a blunted straight needle after nicking the skin with a sharp scalpel. The wires were then pulled through until the couples rested in the center of the leg (half the diameter as measured with a vernier caliper). After insertion the thermocouple wires were soldered to the lead wires of the potentiometer. The legs were then prepared and exposed to cold injury as described in another paper (Pichotka and Lewis, 1949).

In the first experiments a thermocouple was placed in each of the following locations: (1) junction of middle and distal

*From the Department of Pathology, USAF School of Aviation Medicine, Randolph Field, Texas. Submitted for publication February 4, 1952.

thirds of the tibialis anticus (5 to 5½ cm. below upper end of tibia) (*B*, figure 1); (2) distal half of flexor digitorum longus (4 to 5 cm. below upper end of tibia) (*B*, figure 1); (3) middle third of gastrocnemius (2 to 2½ cm below upper end of tibia) (*A*, figure 1).

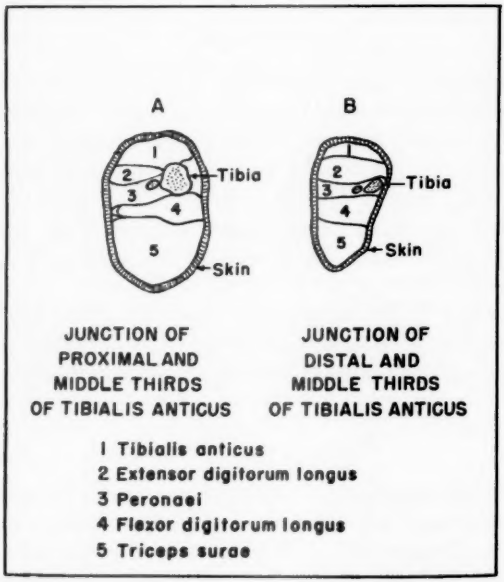


FIG. 1. Drawings of cross sections of rabbit leg showing muscle relationships.

Temperature recordings were made in groups of 6 animals under the following conditions:

A. 0° C. for 30 minutes and thawed in air.*
   —10° C. for 30 minutes and thawed in air.
   —12° C. for 30 minutes and thawed in air.
B. 0° C. for 30 minutes with occlusive tourniquet above knee and thawed in air.†
   —12° C. for 30 minutes with occlusive tourniquet above knee and thawed in air.†

*Room temperature (25 to 30°C).
†Tourniquet applied until end of thawing period.

C. —12° C. for 30 minutes and rewarmed immediately in water at 42° C. for 5 minutes as described in another paper (Pichotka and Lewis, 1951).
—15° C. for 30 minutes and rewarmed immediately in water at 42° C. for 5 minutes.
—20° C. for 30 minutes and rewarmed immediately in water at 42° C. for 8 minutes.

D. —12° C. for 30 minutes and rewarmed with diathermy for 20 minutes as described previously (Lewis, 1951).

Since necrosis occurred first in the distal portion of the tibialis anticus, it was deemed advisable to measure the temperature in the proximal and distal thirds of this muscle simultaneously during exposure to a standard cold injury as follows:

E. —12° C. for 30 minutes.
—12° C. for 30 minutes with occlusive tourniquet above knee.
—12° C. for 30 minutes using animals killed just prior to experiments.

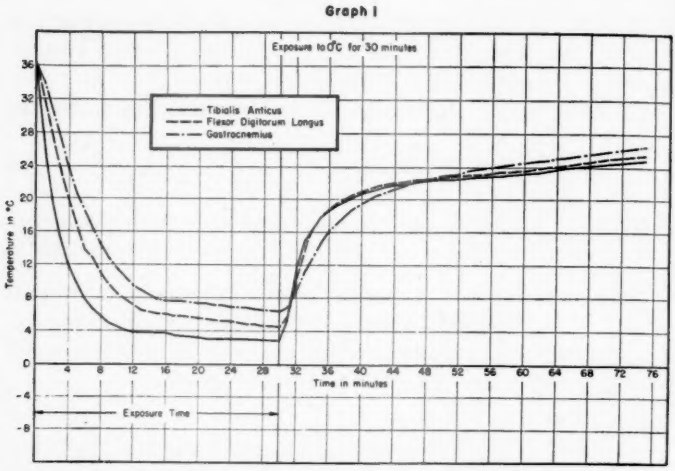
Each graph except No. 13 represents mean values obtained from 6 animals.

Results

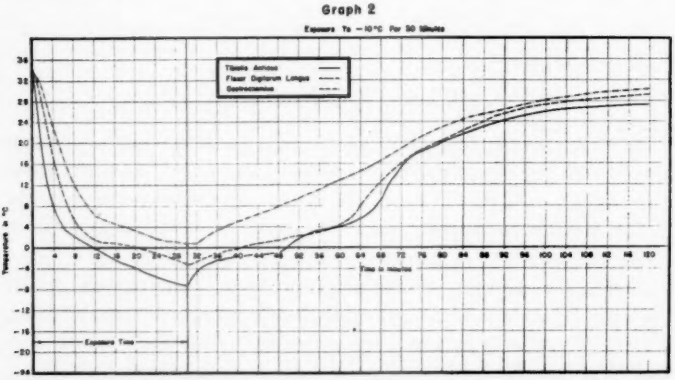
During exposure to a bath temperature of 0° C. for 30 minutes the lowest muscle temperature recorded was +3° C. and this in the tibialis anticus. The second lowest temperature was in the flexor digitorum longus (+4.5° C.), and the temperature of the gastrocnemius was highest (+6.5° C.) (graph 1).

During rewarming in air the muscle temperatures rose rapidly for approximately 14 minutes before tending to level off at about 24° C. They rose in the same order that they had cooled (*i.e.*, the first muscle cooled was the first muscle rewarmed).

During exposure to —10° C. for 30 minutes (graph 2), the temperatures in the tibialis anticus were lowered to —7° C., which is approximately the temperature considered critical for tissue survival by Lake (1917), and which is well below the freezing point of muscle. The temperature in the flexor digitorum longus reached —3° C. which is approximately the freezing point of muscle, whereas the gastrocnemius remained above



GRAPH 1. The muscles cool in this order: (1) tibialis anticus, (2) flexor digitorum longus, (3) gastrocnemius. The rate of cooling follows an exponential curve.



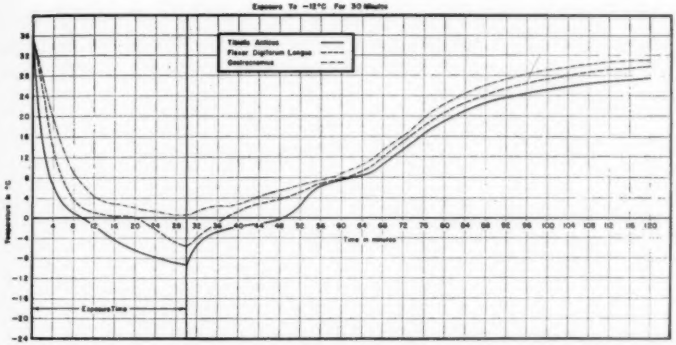
GRAPH 2. The temperature of the tibialis anticus reaches will below freezing that of the flexor digitorum longus extends slightly below the freezing temperature of tissue.

0° C. In the rewarming period the temperature of the tibialis anticus leveled at about —2° to —3° C. for 12 minutes indicating the energy uptake of thawing. Following this, the temperatures in all three muscles gradually increased to reach

24° C. in 1 hour. The order of rewarming was the reverse to the order in which the muscles had cooled.

The shape of the temperature curves is approximately the same for exposure to —12° C. (graph 3). The tibialis anticus

Graph 3

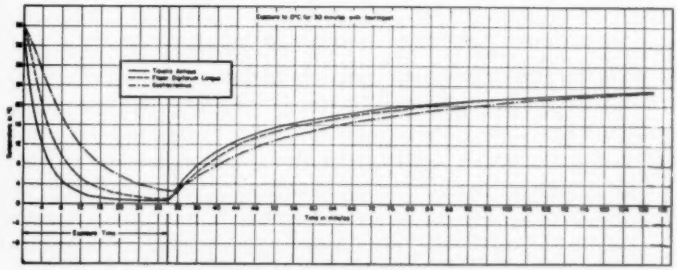


GRAPH 3. The temperature of both the tibialis anticus and flexor digitorum longus extend well below the freezing point of tissue; that of the gastrocnemius remains above 0° C.

and flexor digitorum longus reached freezing temperatures, but the gastrocnemius did not. The temperatures during rewarming are similar to those charted in graph 2.

The results of exposure to 0° and —12° C. with occlusive tourniquets are shown in graphs 4 and 5. In both instances the

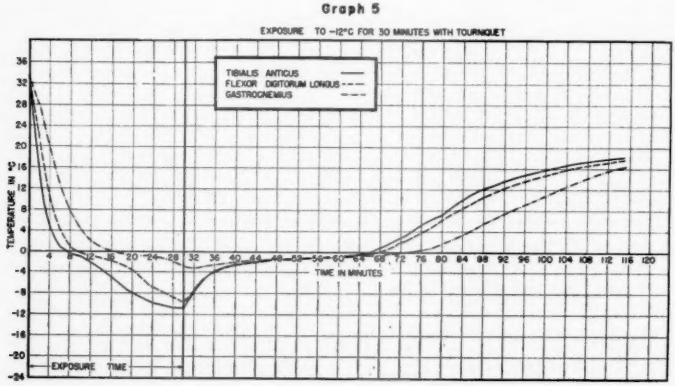
Graph 4



GRAPH 4. All muscles cool more rapidly, become colder, and rewarm more slowly with loss of blood flow (cf. graph 1).

cooling of the muscles was more rapid, lower temperatures were reached than in legs without tourniquets, and rewarming was considerably slower.

Graphs 6, 7, and 8 show the temperature courses during exposure to —12°, —15°, and —20° C., respectively, and during rapid rewarming in water at 42° C. Exposure to —20° C. resulted in temperatures in all muscles well below freezing, and these approached the temperature of the immersion bath. After all three exposures the tibialis anticus rewarmed more rapidly



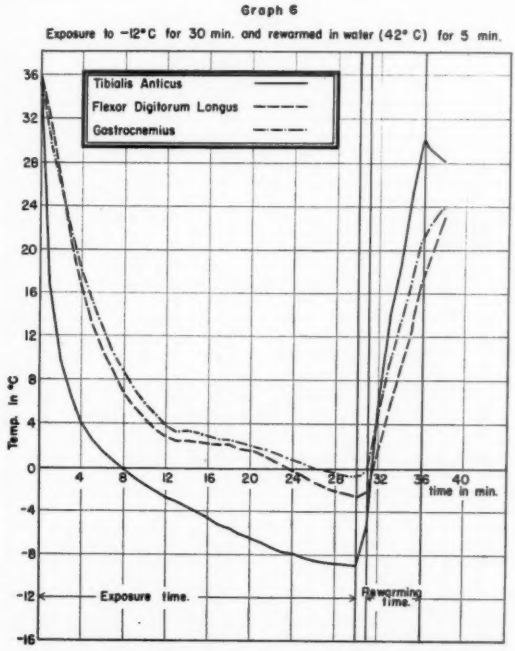
GRAPH 5. All muscles cool more rapidly, become colder, and rewarm more slowly with loss of blood flow (cf. graph 3). Note leveling off of all temperatures at approximately —2° C., reflecting energy uptake due to thawing.

and reached a higher temperature than either the flexor digitorum longus or gastrocnemius. The gastrocnemius after exposure to —12° and —15° C. did not become frozen (or just reached the freezing point) and rewarmed more rapidly than the frozen flexor digitorum longus. Only when the gastrocnemius became frozen (exposure to —20° C. for 30 minutes) did it rewarm more slowly than the flexor digitorum longus.

The temperature curves during exposure to —12° C. for 30 minutes and subsequent rewarming with diathermy are shown in graph 9. These curves differ from those in graph 6 (exposure to —12° C. and rewarmed in 42° C. water) only in a more gradual rewarming of the muscles.

Graphs 10, 11, and 12 show the temperatures in the proximal and distal thirds of the tibialis anticus under three different conditions of exposure to —12° C. for 30 minutes, namely, with normal circulation, with an occlusive tourniquet above the knee, and in dead animals, respectively.

The rate of cooling was greater in legs with tourniquets than in those without, but at the end of 30 minutes' exposure the resultant temperatures were approximately the same. In dead

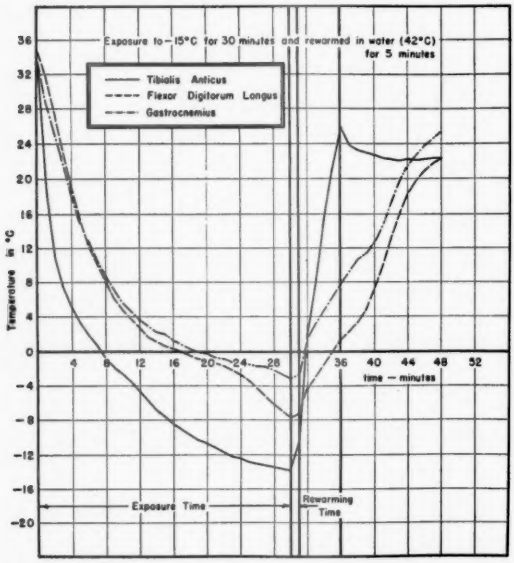


GRAPH 6. All muscles rewarm rapidly but tibialis anticus exceeds the others.

animals the rate of cooling was even greater, and lower temperatures were reached at the end of the 30-minute exposure period than shown in graphs 10 and 11 (graph 12). In all instances the temperatures in the distal third of the tibialis anticus were approximately 4° to 5° C. lower than those in the proximal third.

An interesting phenomenon, similar to that reported by Lewis and Love (1926) in skin, namely, that of supercooling of the muscles, was observed in approximately 10 of 85 legs exposed to various bath temperatures. It was especially frequent during exposures to relatively warm bath temperatures (—8° C.) and was characterized by sudden jumping of the temperatures from below freezing (generally from —3° to —6° C.) to —0.5° C.

Graph 7



GRAPH 7. Temperature curves have the same general shape as those in graph 6.

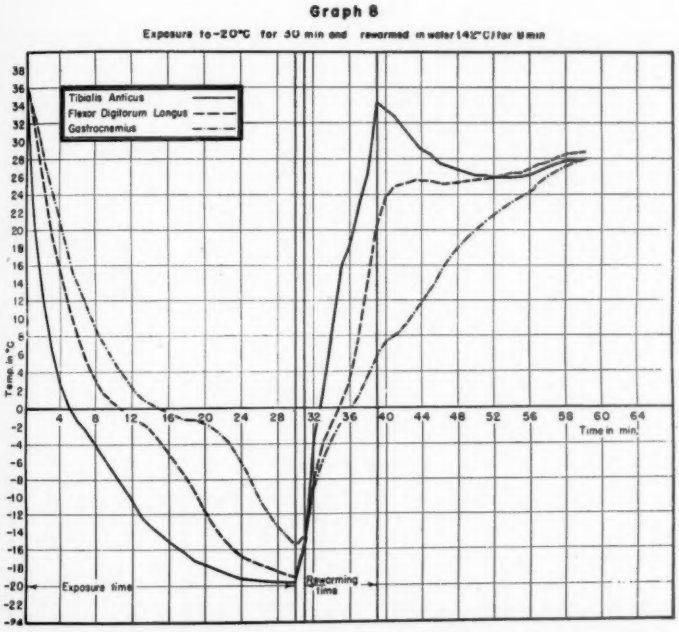
These cases were not used in calculating the mean curves reported in this paper; however, one example of the supercooling phenomenon in a single animal is shown in graph 13.

That this phenomenon was a physiological process and not due to malfunction of the potentiometer was demonstrated by threading two thermocouples through the same needle hole in a

leg and connecting each to a separate potentiometer. This procedure resulted in both instruments registering a jump from a temperature of approximately —5° to —0.5° C.

Discussion

In a previous paper (Pichotka, Lewis, and Freytag, 1951) we showed that, under the conditions of our experiments, exposure of rabbit legs to a bath at —10° and —12° C. for 30 minutes

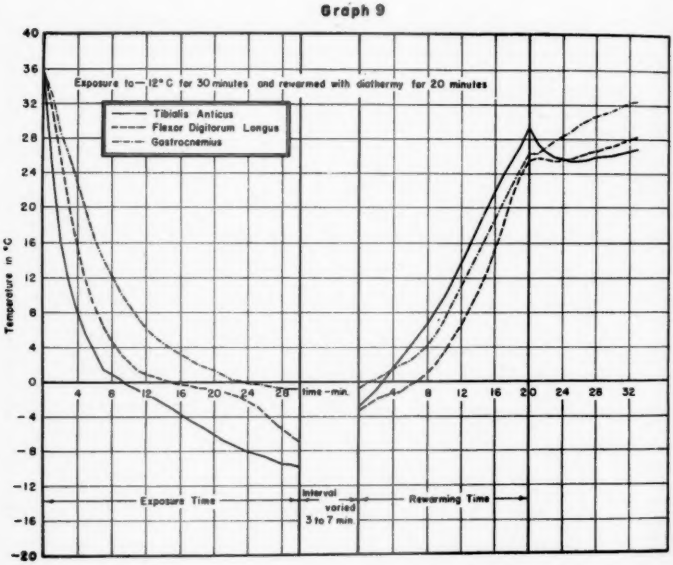


GRAPH 8. Rapid rewarming of all muscles in the same order as their cooling.

produces injuries which are critical for the development of gross muscle necrosis. At these two temperatures 50 and 98 percent of animals, respectively, show macroscopic necrosis of muscle, largely limited to the tibialis anticus and the extensor digitorum

longus which make up a common muscle belly on the anterior aspect of the leg.

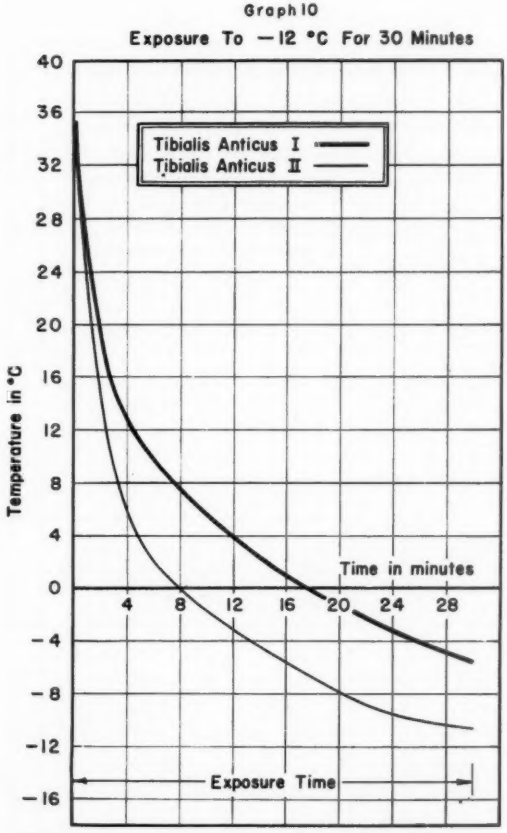
In all instances the order in which the three muscles cool is the same whether with or without a blood supply (*i.e.*, first, tibialis anticus, second, flexor digitorum longus, and third, gastrocnemius). This means that the physical conditions of heat transfer are different for the three muscles, depending on their relative skin surface areas exposed to the bath and their relation to other tissue masses.



GRAPH 9. Rate of muscle rewarming slower but similar to that obtained with water (cf. graph 6). The 3- to 7-minute interval between the end of exposure and the beginning of rewarming was consumed in the preparation of the legs for diathermy treatment.

The flow of blood through the exposed leg hinders the muscle temperatures from approaching the bath temperature during exposure to 0° C. for 30 minutes. This exposure does not result in gross muscle necrosis. This does not mean that exposure to 0° C. for longer periods does not result in necrosis of muscle

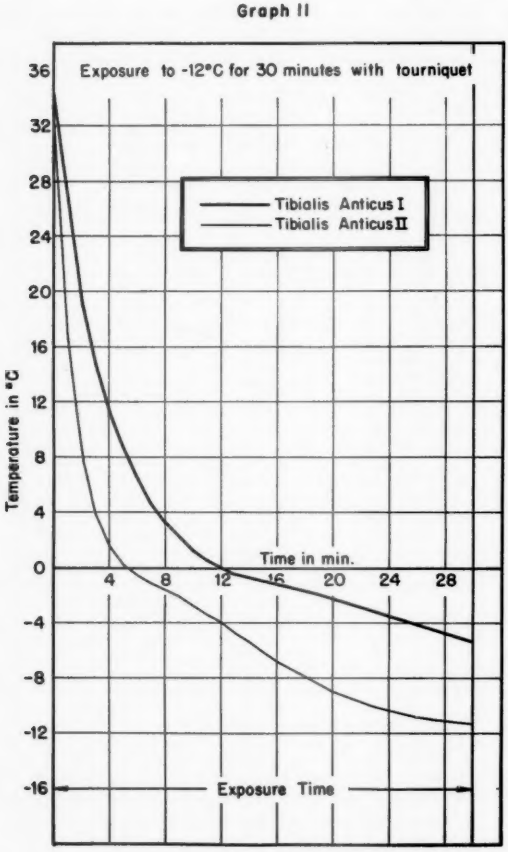
(Lewis and Moen, unpublished). During recovery the temperatures in the muscles rise rapidly to a point some 10° C. below the initial temperatures and thereafter increase very slowly (graph



GRAPH 10. Curve labeled "tibialis anticus I" represents temperatures in proximal one-third of muscle; that labeled "tibialis anticus II," of the distal one-third.

1). This indicates that a residual impairment of circulation or a disturbance of cellular metabolism persists after the exposure period.

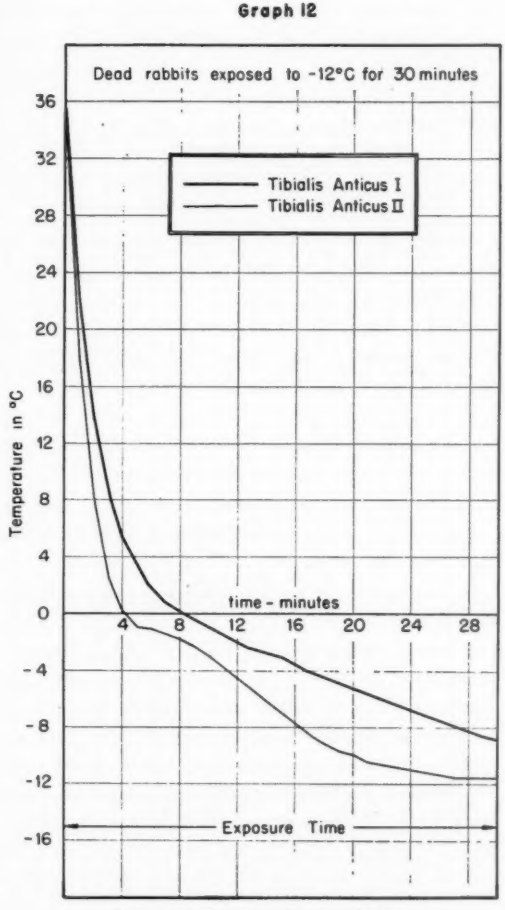
Exposure to —10° C. for 30 minutes results in gross necrosis of small portions of only the tibialis anticus in half the animals (Pichotka, Lewis, and Freytag, 1951), and the others escape



GRAPH 11. Same as for graph 10.

necrosis. The tibialis anticus under these conditions of exposure is the only one that reaches a temperature definitely below the tissue freezing point.

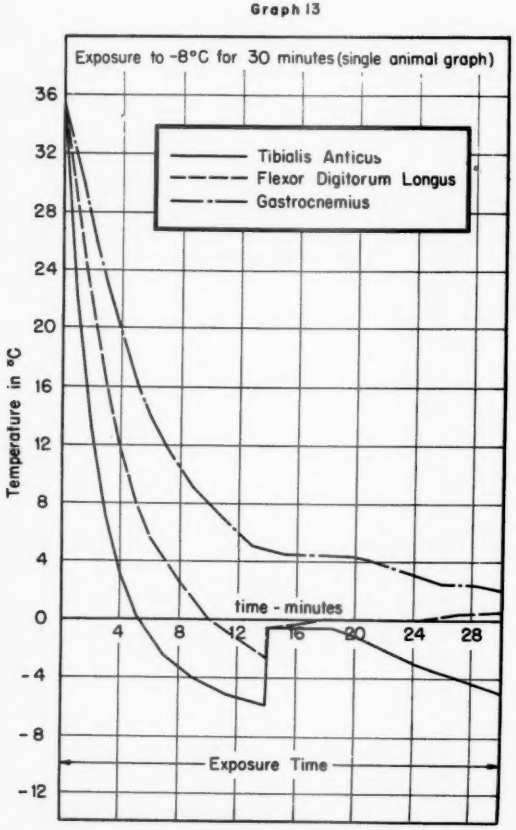
When the bath temperature is lowered to —12° C., the temperature of both the anterior muscle group (tibialis anticus and extensor digitorum longus) and the flexor digitorum longus ex-



GRAPH 12. Same as for graph 10.

tends well below the freezing point of tissue, and both show extensive gangrene. The belly of the gastrocnemius, on the other

hand, remains above freezing temperature and does not become necrotic (graph 3).



GRAPH 13. Temperatures in tibialis anticus and flexor digitorum longus suddenly change from —5.5° and —2.5° C., respectively, to —0.5° C.

Certain aspects of the temperature course in the muscles during rewarming in water at 42° C. and by diathermy following exposure to various degrees of cold injury deserve explanation (graphs 6, 7, 8, and 9). The order and rate of rewarming of

the various muscles are due to their different physical conditions of heat transfer. Factors which govern these are the same as those which control the rate of cooling described previously.

The decrease in the rate of cooling in legs with unimpaired blood flow compared to those with tourniquets (graphs 1 and 4, 3 and 5, 10 and 11) is obviously caused by heat convection incident to blood circulation. Likewise, an intact vascular supply enhances the rewarming process (graphs 1 and 4, 3 and 5).

The more rapid cooling in legs without circulation coincides with the clinical observations with shelter foot in England during World War II (Greene, 1941). Those individuals who sat in deck chairs for hours in air raid shelters with one leg crossed over the other developed symptoms of cold injury in the lower leg due to impairment of circulation from pressure of the chair bars in the popliteal space.

The slower cooling observed in the legs of animals with tourniquets compared to those of dead animals may be explained by the greater conduction of heat from the warm thigh of the former.

It is suggested from graphs 10 and 11 that after tissue freezes (—2° to —3° C.) the role of the vascular system in defending the tissue against further cold injury is negligible, and thereafter the legs behave like dead bodies.

The observation that the distal portion of the tibialis anticus reaches a lower temperature than the proximal portion during cold exposure probably explains the fact that necrosis develops first in the former location. That this difference of temperature in the two areas is not due to variations in blood supply to the two parts of the muscle is demonstrated by the same differences in temperature obtained at these points when occlusive tourniquets are placed above the knee (graph 11). Dead animals show essentially the same difference (graph 12). These observations can be explained only by the relation of the proximal portion of the tibialis anticus to a greater mass of heat-containing and conducting tissue of the leg (*A*, figure 1) compared to that of the distal portion (*B*, figure 1). The cutaneous surface areas of the proximal and distal portions of this muscle exposed to the cold bath are approximately the same and, therefore, not a significant factor.

We cannot adequately explain the fact that the muscle temperature jumps to —0.5° C. during the supercooling phenomenon. A temperature of —0.5° C. is approximately the freezing point of normal saline, whereas —2° to —3° C. seems to be the approximate freezing point of tissue (Pichotka, Lewis, and Freytag, 1951; Lewis and Love, 1926).

It was necessary to determine whether the temperatures recorded were actually those present in the muscle. It was possible that errors resulted from thermal conduction along the 30-gage thermocouple wires since the latter were separated from the cold bath only by the rubber boot covering the leg. for this purpose two thermocouples, one of 30-gage and a second of 36-gage wire, were threaded through the same needle holes in several rabbit legs in the same location and manner as actually used to obtain the data for this paper. In addition to employing smaller sized (36-gage) wire, thermal conduction was further minimized in the finer couple by threading the wire with a needle under the skin for a distance of 1 to 1½ inches from the needle puncture in the leg muscles and closing the small skin incisions. By this method the wire was in close contact with the alcohol bath only after emerging from the skin well above the site of the thermocouple. The two thermocouples were then connected to the same multipoint potentiometer and simultaneous temperature recordings made during exposure of legs to alcohol baths at temperatures of —20°, —15°, and —12° C. In all three instances the two couples registered the same temperature at the end of the 30 minutes' exposure period. At bath temperatures of —20° and —15° C. the finer gage thermocouple registered approximately 0.5° C. warmer during the first 10 and 5 minutes of exposure, respectively. Thereafter, the two recorded the same temperature. Recordings obtained during exposure to —12° C. for 30 minutes were identical with both couples at all times. We, therefore, believe the temperatures given in this paper to have been the actual temperatures within the leg muscles.

Summary

Continuous temperature recordings were made in the following muscle areas, (1) the junction of the middle and distal

thirds of the tibialis anticus, (2) distal half of the flexor digitorum longus, and (3) middle third of the gastrocnemius, during exposure to an alcohol bath at 0°, —10°, —12°, —15°, and —20° C. for 30 minutes and during thawing. In some animals the blood supply to the exposed extremities was cut off during exposure and rewarming by means of tourniquets. Rewarming was accomplished either in air at room temperature, by immersion in a water bath at 42° C., or by short wave diathermy.

During all exposures the temperature in the tibialis anticus was lowered more rapidly and reached lower levels than in the other muscles. The flexor digitorum longus and gastrocnemius were second and third in this respect. This is the same order in which these muscles develop necrosis in the sequence of increasing cold injury (Pichotka, Lewis, and Freytag, 1951).

During exposure to a bath at 0° C. for 30 minutes, muscle temperatures remained a few degrees above zero. (Necrosis of muscle does not develop with this injury.)

The temperature of the tibialis anticus reached approximately —7° C. during exposure to —10° C. for 30 minutes, whereas the other leg muscles remained above or near the freezing temperature of muscle. (Small areas of necrosis develop in the tibialis anticus in approximately 50 percent of animals with this injury.)

Exposure to —12° C. for 30 minutes resulted in temperatures in both the tibialis anticus and flexor digitorum longus well below the freezing point of muscle. (Necrosis in tibialis anticus and flexor digitorum longus occurs in almost all animals with this degree of injury.)

The application of tourniquets increased the rate of cooling and decreased the rate of warming.

Rewarming in water at 42° C. was more effective than with diathermy in raising the muscle temperatures after exposure to cold, and both resulted in more rapid rewarming than exposure to air at room temperature.

Previous experiments (Pichotka, Lewis, and Freytag, 1951) demonstrated that muscle necrosis developed first in the distal half of the tibialis anticus. Reasons for this were obtained in this investigation by recording the temperatures in both the distal and proximal thirds of this muscle during exposure to a

bath at —12° C. for 30 minutes under the following conditions: (1) normal, (2) occlusive tourniquet above knee, and (3) in dead animals. In each situation the temperature in the distal third of the muscle remained approximately 5° C. lower than that of the proximal third. This difference can be explained only by the relationship of the proximal and distal portions of the muscle to different-sized tissue masses of the leg. Differences in blood supply cannot be a factor.

In approximately 10 of 85 instances the phenomenon of supercooling of the muscles was observed during cold exposure and was characterized by a sudden change in temperature from —5° or —6° C. to —0.5° C.

Conclusions

1. In rabbits the tibialis anticus muscle cools more rapidly and reaches lower temperatures than other leg muscles during cold exposure.

2. Macroscopic muscle necrosis does not develop after 30 minutes' exposure to bath temperatures above freezing.

3. For a 30-minute exposure the critical temperature for the development of macroscopic necrosis is —6° or —7° C.

4. An intact blood supply to a limb retards cooling during cold exposure and enhances rewarming after exposure.

5. The temperature course in the various leg muscles is primarily dependent on their relative skin surface areas exposed to the bath and their relationship to other heat-containing tissue masses.

6. Supercooling of muscles occurs occasionally during exposure to cold, and especially during exposure to relatively mild cold baths.

REFERENCES

Greene, R.: Frostbite and kindred ills, *Lancet*, 2:689–693, 1941.

Lake, N. C.: An investigation into the effects of cold upon the body, *Lancet*, 2:557–562, 1917.

Lewis, R. B.: Microwave diathermy treatment of frostbite, *Proc. Soc. Exper. Biol. & Med.*, 78:163–165, 1951.

Lewis, R. B., and Moen, P. W.: unpublished, 1952.

Lewis, T., and Love, W. S.: Vascular reactions of the skin to injury. Part III. Some effects of freezing, of cooling and of warming, *Heart*, 13:27–60, 1926.

Pichotka, J., and Lewis, R. B.: Use of heparin in treatment of experimental frostbite, *Proc. Soc. Exper. Biol. & Med.*, 72:130–136, 1949.

Pichotka, J., and Lewis, R. B.: Effect of rapid and prolonged rewarming on local cold injury, *U. S. Armed Forces M. J.*, 2:1293–1310, 1951.

Pichotka, J., Lewis, R. B., and Freytag, E.: Sequence of increasing local cold injury, *Texas Rep. Biol. & Med.*, 9:613–630, 1951.

MEDICINE IN SHAKESPEARE

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References to things medical are so numerous in Shakespeare's plays that it would take much time merely to recite the most important ones. To note all of them and to interpret their full significance would entail many years of scholarly work. Our chief task shall be to sample them and to arrange them in a few appropriate categories.

Shakespeare's knowledge of his contemporary life was so great that succeeding generations have never ceased to wonder at the capacity of the man. It has been hard for the critic to keep his poise in praising this literary genius. Thus we find Shakespeare has been acclaimed as a classicist, a linguist, a philosopher, a botanist, an astronomer, a lawyer, an anatomist, a neurologist, a physiologist, a psychologist and a physician. Such claims are of course sheer extravagance. There is no evidence that he was professionally trained in any thing. He had curiosity, a prodigious appetite for all kinds of information and a remarkable memory. All this accumulated information he placed at the disposal of his unusual poetic and dramatic ability. This was his genius.

That Shakespeare could make interesting medical allusions no one can doubt. There are over 500 in his works. Whether his information was really profound or whether it was only what one with his gifts might pick up casually, has been much debated. There are those who laud him as a "close student of our learned art," "one of the greatest masters of medicine," "a great physician" and a "teacher of health." Such commentators have I think read more into Shakespeare's plays than is really there. Their interpretations are too liberal and they are made from a twentieth century rather than a sixteenth century point of view. We must not forget Shakespeare was a poet and a dramatist first of all. To achieve a point he made use of any situation or bit of information that suited him. To say,

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"As dear to me as are the ruddy drops
That visit my sad heart"

(Julius Caesar, Act II, Scene 1)

does not prove that the author understood the modern version of the circulation. The phrases are really more satisfactory to the Galenist than they would have been to William Harvey. They do show that the author had some of the current information of his day. His turning this bit of medieval physiology into one of the finest compliments ever paid a wife, that is the magic of Shakespeare.

Neither should one try to project Shakespeare's knowledge of medicine too far into the future. For example, several times Shakespeare uses drugs of one kind or another to put a character to sleep. So Juliet waited for her Romeo. But was this an announcement of the discovery of anesthesia? Or when he says,

"Stand from him, give him air; he'll straight be well,"

was he discoursing on a modern form of open air treatment? Such deductions have been made but they seem forced. Such claims are more in the minds of the critics than ever they were in the mind of Shakespeare. The author did have an extraordinarily wide knowledge of the medical lore of his day. Medicine has always been forward looking, even in the darkest of ages. Hardly a statement of any kind could be made that could not be tortured into a prophesy if the critic were diligent enough.

One cannot help wondering what was the source of all this medical information. He must have listened well and to all sorts of people. Quite likely he did read a certain amount of medical literature and conversed with physicians. It must not be forgotten that his daughter Susanna married John Hall, a physician. Dr. Hall seems to have been an accomplished man and was the author of a well known book on "Select Observations on English Bodies and Cures Empirical." We know that Shakespeare lived with his son-in-law and daughter from 1608 until his death in 1616. Whether this explains some of the poet's familiarity with physic is of course conjecture. It would be of interest to compare the plays during this period with the earlier ones.

Granted that Shakespeare could speak the language of Aesculapius well enough to enter medical circles, the real question is, from the standpoint of science and art, how much medicine did he really know. We may never be able to answer this to our own satisfaction but it can be approached by learning Shakespeare's attitude toward the practitioners of the healing art and by evaluating the medical information he puts on display.

Some six branches of medical practice are represented in Shakespeare's characters; the physician, the surgeon, the apothecary, the tooth-drawer, the mid-wife and the nurse. Of these the physician easily outranks the others as a class. The reason for this is well known historically. The clergical physicians after practicing surgery for many centuries were deprived of that right by the decree of Innocent III in 1215. They circumvented the papal bull by having their barbers do any work which involved the shedding of blood. Thus arose the barber-surgeons. But until 1635 it was impossible for a barber-surgeon to "do any great operation" except in the presence of a "learned physician." In Shakespeare's day the surgeon was then poorly educated and decidedly of a low caste. He is so pictured by the dramatist.

It is hard to decide just how much respect Shakespeare really had for physicians. Certainly he knew a lot about them and their practices. Some of those he pictures are charlatans and rogues, while others are represented as men of exalted virtue. The truth may be that he cares neither one way or another about them, but merely used them for his dramatic purpose. At any rate there is no constant casting of aspersions at physicians, such as we find in Moliere, although there are many sly digs at their weaknesses.

England was cursed during the Elizabethan period with many quacks, charlatans, mountebanks and medical rogues. They are not unknown today. Shakespeare could hardly have pictured the life of his time or any previous one without many references to them.

Let us first refer to those physicians whose behaviour gives us pride in their profession.

Cornelius in Cymbeline is a pharmacologist to whom we shall refer again, but he is also an honest doctor. He is com-

manded by the queen to furnish her with an assortment of most deadly poisons. Suspicious of her motives Cornelius says,

> "I beseech your grace, without offense—my conscience bids we ask—wherefore you have commanded of me these most poisonous compounds?"
>
> (Act I, Scene 5)

True to his oath as a physician and unsatisfied with her reply Cornelius says aside,

> "I do not like her. She doth think she has strange lingering poisons; I do know her spirit and will not trust one of her malice with a drug of such dam'd nature."

Several medical peers were known in England during the days of Queen Elizabeth. Of particular note and renown were the Marquis of Dorchester, and Edmund, Earle of Darby. Shakespeare also recognized that a medical education might be an honor to a man of any rank. Thus Cerimon, the Lord of Ephesus, a character in Pericles was a student of physic and in his mouth the dramatist puts these words which express high medical ideals in the most exalted language.

Cerimon: "I held it ever,
Virtue and cunning were endowments greater
Than nobleness and riches; careless heirs
May the two latter darken and expend;
But immortality attends the former,
Making a man a god. Tis known, I ever
Have studied physic, through which secret art,
By turning o'er authorities, I have
(Together with my practice) made familiar
To me and to my aid the blest infusions
That dwell in vegetatives, in metals, stones;
And I can speak of the disturbances
That nature works, and of her cures; which doth give me
A more content in course of true delight,
Than to be thirsty after tottering honour,
Or tie my treasure up in silken bags,
To please the fool and death."

(Act III, Scene 2)

The Scotch doctor in Macbeth is a rather careful timid man, but his character is sound and he refuses to prostitute his talents to ignorance or to capitalize on a direful situation as a less noble physican might have done. It will be recalled that he had been summoned by Lady Macbeth's waiting maid because of the Queen's nightwalking and strange behaviour, which were expressions of her remorse over the murder of Duncan.

She seems to wash her hands. She cries, "Out damned spot! Out I say." "Will these hands ne'er be clean?" "All the perfumes of Arabia will not sweeten this little hand."

The doctor watches and hears and understands. "This disease is beyond my practice," he says, "more needs she the divine than the physician."

A little later Macbeth makes inquiry as to how his lady is. The doctor replies,

> "Not so sick, my lord,
> As she is troubled with thick-coming fancies,
> That keep her from her rest."
>
> "Cure her of that," storms Macbeth,
> "Canst thou not minister to a mind diseased
> Pluck from the memory a rooted sorrow,
> Raze out the written tablets of the brain,
> And with some sweet oblivious antidote
> Cleanse the stuff'd bosom of that perilous stuff
> Which weighs upon the heart?"
>
> (Act V, Scene 3)

Even today we cannot meet Macbeth's demands to our satisfaction. The doctor in Shakespeare's time could only reply with gentleness and a humble sense of his own inability,

> "Therein the patient
> Must minister to himself."

Macbeth, himself tottering on the brink of insanity, screams

> "Throw physic to the dogs, I'll have none of it."

It is small wonder that the doctor is glad to leave these terrifying scenes and the guilty situations in which his art is helpless and that he says most humanly,

> "Were I from Dunsinane away and clear
> Profit again should hardly draw me here."

On the other hand the doctor in Lear seems to be a master of his psychiatric art. His care of the demented king has always been praised as humane and wise. Treatment of the insane during the Elizabethan period was a thing of horror and it remained so until our own day. Rosalind in teasing Orlando in "As you Like It" refers to the cruel mediaeval treatment of the insane.

> Rosalind.
> "Love is merely a madness; and I tell you,
> deserves as well a dark house and a whip as
> mad men do."
>
> (Act III, Scene 2)

In the face of this, the treatment given by the doctor to poor King Lear is very advanced. Cordelia asks if there is aught that man's wisdom can do in restoring her father's bereaved sense. The doctor replies,

> "There is a means, madam,
> Our foster-nurse of nature is repose,
> The which he lacks; *that*, to provide in *him*
> Are many simples operative, whose power
> Will close the eye of anguish."
>
> (Act IV, Scene 4)

The doctor puts his theories into practice. The king is made to sleep and later allowed to waken to the sound of music, his senses fully restored.

Shakespeare's regard for medicine may also be illustrated by "All's Well that Ends Well," which may be said to have a medical plot. Helena is the daughter of the fictitious but celebrated physician Gerard of Narbon. His character is described as one,

> "Whose skill was almost as great as his honesty; had it
> stretched so far, would have made nature immortal,
> and death should have play for lack of work."
>
> (Act I, Scene 1)

This great man had left to Helena his dearest possession, a prescription and appliance for the cure of fistula. This she proposes to use in order to save the life of the French King. The king is finally convinced that she may have a worthwhile treatment and submits to her ministrations. Of course he recovers.

Authorities are not agreed as to the nature of the fistula. It has been assumed to be a chest abscess, but old Lord Lafeu speaks of it as being "nortorious." It may have been rectal, notorious referring to the strong olfactory evidence of its nature.

In Dr. Caius of the "Merry Wives of Windsor" Shakespeare *caricatures* the pretensions of a French physician who has come to England to make his fortune. Dr. Caius is supposed to be a picture of Turquet de Magern who became Sir Theodore de Magerne after having crossed the channel, and having become a fashionable doctor and finally a physician to the court. Why Shakespeare called him Caius is entirely unknown. Certainly there is no relation to Dr. Caius, one of the founders of Caius and Gonville College. Dame Quickly labels Dr. Caius a combination of a fool and a physician. On the stage he supplied the comedy role with a strong French brogue. He was dubbed "Belly Stale," "A Castalian King Urinal" and "Monsieur Mockwater." He threatens to *remove* the emarculate *testicles* of Sir Hugh Evans whose interference in a love affair he strongly resents. Only one person stands up for this caricature of a medical man, and this is mine host of the Garter Inn who praises him in the immortal words,

> "Shall I lose my doctor? No. He
> gives me the potions and the motions."
>
> (Act III, Scene 1)

The only actual living doctor that Shakespeare has among his characters is Dr. Butts in Henry VIII. He appears here as a courtier defending Cranmer and not as a physician. In the famous Holbein picture which is in the Hall of the Barber's Company, Dr. Butts is at the right of the King. He was knighted and received rich grants of abbey lands.

Of course there are references to Aesculapius, Hippocrates and Galen. In the "Merry Wives of Windsor" Mine Host calls Dr. Caius "My Aesculapius," "My Galen." Sir Hugh is not so appreciative of the French doctor whom he describes as having no knowledge of Hippocrates or Galen. In "All's Well that Ends WWell" Helena cures the King after he has been given up by the practitioners both of Galen and Paracelsus. Shakespeare was thus apparently familiar with these two schools of thought.

But the author also used the name of Galen for one of his jibes at the profession. In Coriolanus the spirits of the tribune Menenius are much raised by a letter from Coriolanus who is coming home after his great victory at Corioli.

> "A letter for me! it gives me an estate of seven years' health; in which time I will make a lip at the physician; the most sovereign prescription in Galen is but empiricutic, and to this preservative, of no better report than a horse drench."
>
> (Act II, Scene 1)

Just how much spleen there is in this passage is hard to say.

Dr. Pinch in the "Comedy of Errors" is an excellent picture of a mountebank and humbug who masquerades as a doctor. It will be remembered that Antipholus of Ephesus and Antipholus of Syracuse, really identical twins, have gotten so mixed up that Adriana believes her husband Antipholus of Ephesus has gone mad. She calls on Dr. Pinch who feels the patient's pulse and then proceeds to remove the devil in him by holy prayers. Pinch is really an unadulterated imposter and later is graphically described as

> "A hungry lean faced villain
> A mere anatomy, a mountebank,
> A threadbare juggler and a fortune teller
> A needy, hollow-eyed, sharp looking wretch
> A living dead man."
>
> (Act V, Scene 1)

Friar Laurence in Romeo and Juliet is something of a cross between a doctor and an apothecary. He administers a drug which stops all signs of life in Juliet for two and forty hours. His effort to spare Romeo and Juliet from their hard *fate* of course wins our sympathy although we know he possessed no such powers as those he advertised.

If we may judge from the famous passage in Romeo and Juliet the apothecary and his calling were not very highly esteemed in the Elizabethan period. Romeo fails to hear from Friar Laurence but, Bathazar does inform him of Juliet's supposed death and interment in Capulet's monument. In despair Romeo determines on suicide and goes to a poor apothecary's

shop for a fatal drug. In a masterpiece of description the man and his shop stand vividly before us:

> "I do remember an apothecary,
> And hereabouts a'dwells, which late I noted
> In tattered weeds, with overwhelming brows,
> Culling of simples; Meager were his looks:
> Sharp misery had worn him to the bones:
> And in his needy shop a tortoise hung,
> An alligator stuff'd and other skins
> Of ill-shaped fishes: and about him his shelves
> A beggardly account of empty boxes,
> Green earthern pots, bladders and must seeds
> Remnants of packthread and old cakes of roses
> Were thinly scattered, to make up a show."
>
> (Act V, Scene 1)

The apothecary confesses that he has the mortal drugs that Romeo desires, but that Mantua's laws are death to one who even utters their names. Forty ducats however wins him over and he excuses himself in the words, so often heard throughout all time,

> "My poverty, but not my will, consents."

Shakespeare and the Circulation

Certainly Shakespeare appreciated the importance of the heart and circulation of the blood. It may be that we can go so far as to say that medical men of the day were conscious that discoveries of importance were in the offing, and that Shakespeare was cognizant of that feeling. But probably we cannot really go even that far in speculation. There is no reason for believing that the poet knew Harvey and learned anything from such an acquaintance. Harvey first announced his theories in 1613 and by this time Shakespeare was through his work. Careful examination of the passages having to do with the circulation will show that as astute and clever as they are, they still are only the current information of the time, that is strictly Galenical. This conception, erroneous as it proved to be, Shakespeare nevertheless seems to have mastered.

In Coriolanus is one of the most interesting of these passages.

The citizens of Rome have mutinied against the power of the patricians, Caius Martius in particular. Menenius Agrippa, a friend of Caius, strives to convince them that their ills are not due to the patricians but that the latter have really tried to extend charitable care to the people. To illustrate his point he elaborates an old fable that goes back to antiquity.

"There was a time when all the body's members
Rebell'd against the belly; thus accused it;
That only like a gulf it did remain
I' the midst of the body, idle and inactive,
Still cupboarding the viand, never bearing
Like labour with the rest; where the other instruments
Did see and hear, devise, instruct, walk, feel,
And, mutually participate, did minister
Unto the appetite and affection common
Of the whole body. The belly answered—

* * * * * *

'True is it, my incorporate friends,' quoth he,
'That I receive the general food at first,
Which you do live upon; and fit it is,
Because I am the store-house and the shop
Of the whole body: but, if you do remember,
I SEND IT THROUGH THE RIVERS OF YOUR BLOOD,
EVEN TO THE COURT? THE HEART? TO THE SEAT O' THE BRAIN:
And, through the cranks and offices of man,
The strongest nerves and small inferior veins
From me receive that natural competency
Whereby they live;

* * * * * *

(Act I, Scene 1)

In "Measure for Measure" the deputy Angelo, the soul of morality until temptation comes to him, considers the seduction of the virtuous Isabello. Such action is really foreign to his nature and he exclaims:

"O heavens!
Why does my blood thus muster to my heart,
Making both it unable for itself,
And dispossessing all my other parts
Of necessary fitness."

(Act II, Scene 4)

This might be said to be a fairly good poetical description of congestive heart failure.

Malvolio in Twelfth Night is aware that cross-gartering, that is wearing the agrters in crisscross fashion around the leg,

> "does make some obstruction in the blood."
>
> (Act III, Scene 4)

Blocking the return of the blood by pressure on a vein was one of Harvey's proofs of the circulation, but the actual fact was known of course centuries before. It was the interpretation of Harvey that was new.

The old doctrine that both health and disease depended on the quality of the humors is background for allusions to the healthful and unhealthful properties of the blood. There are several references to the life of the blood. Prince Henry says of the dying King John,

> "The life of all his blood
> Is touched corruptibly."
>
> (Act V, Scene 7)

In the "Life of King John," King Philip asks King John how he dares call himself a King so long as a

> "living blood doth in these temples beat"
>
> (Act II, Scene 1)

The reference is to the young Arthur who was the real heir to the English throne. In the same play Cardinal Pandulph also speaks of the life of the blood. King John can have no rest

> "That whiles warm life plays in that infant's veins."

Falstaff in King Henry IV, part 2, gives a very wonderful discourse on the influence of sherry on the circulation based on Galen.

> "A good sherry sack hath a two fold operation in it. It ascends me into the brain; dries me there all the foolish and dull and crudy vapours which environ it; makes it apprehensive, quick, forgetive, full of nimble, fiery and delectible shapes. The second property of your excellent sherry is the warming of the blood; which, before cold and settled left the liver white and pale, which is the badge of pusillanimity and cowardice; but the sherry warms it and makes it course from the inwards to the parts

extreme: . . . and then the vital commoners and inland petty spirits *muster* we all to their captain, the heart, who great and puffed up with this retinue, doth any deed of courage; and this valour comes of sherry."

(Act IV, Scene 3)

The same idea is also expressed by Sir Tobey Belch in Twelfth Night who hasn't a very high regard for the oldish lover Andrew.

> "For Andrew, if he were opened,
> and you find so much blood in his liver as
> will clog the foot of a flea, I'll eat the
> rest of the anatomy."
>
> (Act III, Scene 2)

Pharmacology and Therapeutics

In Act I, scene 5 of Cymbaline is an interesting bit of pharmacology. We have already quoted a part of this scene to illustrate the character of a conscientious doctor. Shakespeare seems quite familiar with the general way that toxicity experiments are carried out, and describes them in language quite understandable today.

Cymbaline's queen wishes to be rid of her husband's daughter Imogene and chooses poison for her purpose. These she expects to get from Dr. Cornelius and the scene opens as he enters with the drugs.

> Cornelius; Pleaseth your highness, ay; here they are, madam;
> But I beseech your grace, without offence—
> My conscience bids me ask—wherefore you have
> Commanded of me these most poisonous compounds,
> Which are the movers of a languishing death,
> But though *slowly*, deadly.
>
> The Queen replies: I wonder, Doctor,
> Thou ask'st me such a question. Have I not been
> Thy pupil long? Hast thou not learned me how
> To make perfumes? distil? preserve? yea, so
> That our great King himself doth woo me oft
> For my confections? Having thus far proceeded,
> Unless thou thinks't me devilish—is't not meet
> That I did amplify my judgment in
> Other conclusions? I will try the forces

> Of these thy compounds on such creatures as
> We count not worth hanging, but none human,
> To try the vigour of them and apply
> Allayments to their act, and by them gather
> Their several virtues and effects.

Cornelius is not much of an experimenter:

> Cornelius; Your highness
> Shall from this practice but make hard your heart;
> Besides, the seeing these effects will be
> Both noisome and infectious.
>
> Aside, Cornelius continues;
> I do not like her. She doth think she has
> Strange lingering poisons; I do know her spirit,
> And will not trust one of her malice with
> A drug of such damn'd nature. Those she has
> Will stupefy and dull the sense awhile;
> Which first, perchance she'll prove on cats and dogs,
> Then afterward up higher.

The queen even prepared to poison Cymbeline and on her death bed confessed to Doctor Cornelius that

> "she had
> For you a mortal mineral; which, being took,
> Should by the minute feed on life and lingering
> By inches waste you;

This sounds like arsenic which was king of all poisons until its detection was made possible by the Marsh test.

The many references to the use of poisons might well be expected in tragic literature and we know that their use was actually common until the development of chemistry in the nineteenth century made detection possible. Shakespeare very evidently had looked into the subject rather carefully and was quite familiar with the poison lore of his day.

In Hamlet, the ghost of the King tells how he was poisoned by his brother, the uncle of Hamlet, the Prince of Denmark.

> "Sleeping within my orchard,
> My custom always of the afternoon,
> Upon my secure hour thy uncle stole,
> With juice of cursed hebenon in a vial,
> And in the porches of mine ear did pour

The leperous distillment; whose effect
Holds such an enmity with the blood of man
That swift as quicksilver it courses through
The natural gates and alleys of the body;
And with a sudden vigour it doth posset
And curd, like eager droppings into milk,
The thin and wholesome blood; so did it mine;
And a most instant tetter bark'd about,
Most lazar-like, with vile and loathsome crust,
All my smooth body."

(Act I, Scene 5)

Inspired by this Dr. Macht at Baltimore tested out absorption by the ear drum and found that certain drugs could enter the blood stream by that route in rather large quantities.

In Macbeth, after Macbeth and Banquo have had their meeting with the witches, Banquo says:

"Were such things here as we do speak about?
Or have we eaten on the insane root
That takes the reason prisoner?"

(Act I, Scene 3)

This is a reference to henbane or hyoscyamus a drug believed to induce madness.

Certainly one of the most interesting scenes in all Shakespeare is that of the witches in Act IV of Macbeth. These ugly, foul, grotesque figures, as they dance around the bubbling cauldron are the epitome of malice and meanness, but nevertheless to the superstitious mind they are possessed of terrible power. What shall go into the pot to make the terrible brew? All the objects that for time immemorial have had magical powers. Here they are:

"Round about the cauldron go;
In the poison'd entrails throw.
Toad, that under cold stone
Days and nights has thirty one
Swelter'd venom got,
Boil thou first i' the charmed pot.

Fillet of a fenny snake,
In the cauldron boil and bake;
Eye of newt and toe of frog,

Wool of bat and tongue of dog,
Adders fork and blind-worm's sting,
Lizard's leg and howlet's wing,

Scale of dragon, tooth of wolf,
Witches' mummy, maw and gulf
Of the ravin'd salt sea shark,
Root of hemlock digg'd i' the dark,
Liver of blaspheming Jew,
Gall of goat and slips of yew
Sliver'd in the moon's eclipse,
Nose of Turk and Tartar's lips,
Finger of birth-strangled babe
Ditch delivered by a drab,
Make the gruel thick and slab;
Add thereto a tiger's chaudron,
For the ingredients of our cauldron

Cool it with a baboon's blood,
Then the charm is firm and good.

(Act IV, Scene 1)

Where did Shakespeare ever get such a list of terrible nauseating things? We cannot discount his own imaginings, but they remind the medical man of the old theriacs. Medical concoctions with some of the same ingredients were still in vogue in the days of Shakespeare. The first Pharmacopea was published in 1618, and in this are 91 animal products recommended for various diseases. Often the prescription contained as many as a dozen or more ingredients and the famous Mithridatic devised by Galen had an even hundred. Among the things recommended by the medical men of Shakespeare's day were: teeth, claws, hoofs and bones of various animals, snake skins, scorpions, wood lice; bones of an executed criminal; human urine, menstrual fluid and human feces. It is probable that every item mentioned in the witches scene can be duplicated in medical literature. It seems to me highly probable that Shakespeare may have read some of such literature. The witches brew is a poetical version of a medical prescription of the day. As a matter of fact such bizarre remedies persisted in the Pharmacopea until 1746, when they disappeared due to the work of Heberden.

As for the use of alcohol, Shakespeare's opinions are about as definite as those of the medical profession today, some for,

some against. Falstaff in King Henry IV, Part 2 extols the virtues of sherry-sack. He says of Prince Henry:

"A man cannot make him laugh; but that's no marvel, he drinks no wine. There's never none of these demure boys come to any proof; for thin drink doth so over-cool their blood, and making many fishmeals, that they fall into a kind of male green-sickness; and then when they marry, they get wenches; they are generally fools and cowards."

(Act IV, Scene 3)

The Porter in Macbeth however gives a more vivid but less enthusiastic discourse on the effects of drink. The talkative fellow remarks that liquor is a great provoker of three things, and on begging to be informed Macduff learns what they are:

Marry, sir, nose-painting, sleep and urine.
Lechery, sir, it provokes and unprovokes; it
provokes the desire, but it takes away the
performance; therefore much drink may be said to be an
equivocator with lechery; it makes him and it mars
him; it sets him on and it takes him off; it
persuades him and disheartens him; makes him stand
to and not stand to; in conclusion equivocates him
in a sleep, and giving him the lie, leaves him.

(Act III, Scene 3)

On the whole Shakespeare seems not very favorable to liquor. In Othello, he has Cassius say:

"O thou invisible spirit of wine: if thou hast no name to be known by,
Let us call thee, Devil.
Oh God, that man should put an enemy in his mouth to steal his
brains away,
I dare not trust my weakness with any more."

But Old Adam in "As You Like It" has no doubts as to what made possible his hearty old age. It was youthful temperance and chastity. Would that we all might say,

"Though I look old, yet am I strong and lusty
For in my youth I never did apply
Hot and rebellious liquors in my blood
Nor did I with unbashful forehead woo
The means of weakness and debility.
Therefore my age is as a hoary winter,
Frosty but kindly."

(Act II, Scene 3)

Many drugs are mentioned by name. Macbeth asks the Doctor,

> "What rhubarb, senna or what purgative drug,
> Would scour these English hence?"
>
> (Act V, Scene 4)

In "Much Ado About Nothing" is a reference to Carduus Benedictus or holy thistle, which until 1693, was reputed to cure everything from pleurisy to worms.

Mandrake was a wonderful soporific throughout Shakespeare's day. He refers to this property of the drug in two of the most beautiful passages in all the plays.

Cleopatra asks Charmian for a drink of mandragora, and the maid asks "Why, madam?" to which the queen replies:

> "That I might sleep out this great gap of time
> My Antony is away."
>
> (Act I, Scene 5)

Othello refers to the poppy and mandrake in much the same sense in the exquisite lines:

> "Nor poppy nor mandragora
> Nor all the drowsy syrups of the world
> Shall ever medicine thee to that sweet sleep
> Which thou owd'st yesterday."
>
> (Act III, Scene 3)

Syphilis

Syphilis became known in Europe only one hundred years before Shakespeare's day and it was still attracting both public and professional attention because of its newness and its terrible ravages. Shakespeare had a considerable knowledge of the disease and he makes many references to it. He refers to it as "the malady of France," "the rotten disease of the south" and the "Neopolitan bone ache."

The crusts and tubercles about the forehead, temples and behind the ears often formed the figure of a crown. It was to this that Quince refers in Midsummer's Nights Dream:

> "Some of your French crowns have no hair at all."
>
> (Act I, Scene 2)

Timon was very justly incensed against Athens whose citizens treated him well so long as he had money, but who entirely ignored him when his finances failed. In his anger he utters most excellent curses against his erstwhile friends.

> Thou cold sciatica
> Cripple our senators, that their limbs may halt
> As lamely as their manners. Lust and liberty
> Creep in the minds and marrow of our youth
> That gainst the stream of virtue they may strive,
> And drown themselves in riot. Itches, blains,
> Sow all the Athenian bosoms, and their crop
> Be general leprosy. Breath infect breath,
> That their society, as their friendship, may
> Be merely poison.
>
> (Act IV, Scene 1)

In scene 3 he counsels the harlot Timandra to

> "Give them diseases, leaving with thee their lust.
> Make use of thy salt hours; season the slaves
> For tubs and baths; bring down rose-cheeked youth
> To the tub-fast and the diet."

That the poet understood the ravages of the French disease is evident from Timon's further sarcastic advice to Timandra and Phrynia, which is an accurate and excellent description of tertiary syphilis:

> "Consumptions sow
> In hollow bones of man; strike their sharp shins,
> And mar men's spurring. Crack the lawyer's voice,
> That he may never more false title plead,
> Nor sound his quillets shrilly; hoar the flamen,
> That scolds against the quality of flesh
> And not believes himself; down with the nose,
> Down with it flat; take the bridge quite away
> * * * * * *
> make curl'd-pate ruffians bald;
> And let the unscarr'd braggarts of the war
> Derive some pain from you; plague all;

Obstetrics and Gynecology

Shakespeare was familiar with much of the medical lore of his day that had to do with obstetrics and gynecology. Every

father naturally picks up some such information but the author seems to have accumulated somewhat more than might be expected of the average layman.

A belief in prenatal influences was current then as now, emphasis being placed however mostly on the effect of emotions in the mother. Thus in Henry VI, Part III, Queen Elizabeth says,

> "And I the rather wean me from despair
> For love of Edward's offspring in my womb
> This is it that makes me bridle passion,
> And bear with mildness my misfortune's cross,
> Lest with sighs or tears I blast or drown
> King Edward's fruit, true heir to England's crown."
>
> (Act IV, Scene 4)

There are two well known references to Caesarian section. The witches had told Macbeth that he led a charmed life, and that he need never fear one born of woman. This belief supported him in all his villainies and led him to boast:

> "I bear a charmed life which must not yield
> To one of woman born."
>
> (Act V, Scene 3)

But in the battle Macduff replies to the vain boaster:

> "Despair thy charm
> And let the angel whom thou still hast served
> Tell thee, Macduff was from his mother's womb
> Untimely ripp'd."

Again in Cymbaline we have the lines:

> "Lucina lent me not her aid
> But took me in her throes,
> That from me was Posthumus ripp'd
> Came crying 'mongst his foes
> A thing of pity."
>
> (Act V, Scene 4)

Lucina was the goddess of childbirth.

The first cry of the infant is referred to in the preceding lines, but much more clearly in a passage from Lear.

> "Thou knowist the first time that we smell the air
> We maul and cry.

When we are born we cry that we are come
To this great stage of fools."

(Act IV, Scene 6)

Although there is no reference to death in child-bed there are many allusions to difficult labour. In Henry VIII the suffering of Anne Boleyn is described:

" . . . the Queen's in labour
They say in great extremity, and feared
She'll with the labour end."

(Act V, Scene 1)

The result of her agony was, much to the disgust of the voluptuous Henry, a daughter, the future Queen Elizabeth.

The Duke of Gloucester, a monster in shape and mien who later became Richard the Third, was ushered into the world by his mother's ill omened and difficult labour. It is described in Henry VI, Part III.

"Thy mother felt more than a mother's pain,
And yet brought forth less than a mother's hope."

(Act V, Scene 6)

Later Gloucester describes his own birth.

"For I have often heard my mother say
I came into the world with my legs forward.
The midwife wondered and the women cried
'O Jesus bless us he is born with teeth'
And so I was. Which plainly signified
That I should growl and bite and play the dog."

(Act V, Scene 6)

Throughout all the ages much trust in certain quarters has always been placed in Aphrodisiace. Of all the characters of Shakespeare, Falstaff should be well informed of such matters and we do have this interesting invocation of his in the Merry Wives of Windsor.

"Let the sky reign potatoes; let it thunder to
the tune of "Green Sleeves," hail kissing comfits, snow
eringoes; let there come a tempest of provocation, I
will shelter me here."

(Act V, Scene 5)

Potatoes were sweet potatoes and eringo was sea holly, both famed for their stimulating effects. The love song "Green Sleeves" is still known.

Midwives are referred to rather frequently but not always too respectfully. In Twelfth Night Marcius says,

> "Nay but say true, does it work upon him?"

and Sir Toby replies;

> "Like aqua vitae on a midwife."
>
> (Act IV, Scene 4)

The character of this species remained true in a literary way at least until the time of Sarah Gamp immortalized by Dickens. Many more obstetrical references might be given if time permitted. So many are there that Dr. Field says "Obstetrics was Shakespeare's favorite branch of the profession."

Hygienic Advice

Literature of all ages has many adages and bits of homely advice that are in line with the best hygienic advice that medicine can give. Quite a number of these appear in Shakespeare. The beneficial effect of happiness is touched on in the Taming of the Shrew.

Christopher Sly, a common drunk, is discovered by a real Lord, who dresses him up in finery, and surrounds him with servants who make believe that poor Christopher is a real lord himself who has dreamed and slept away the past 15 years. Players are brought to give him cheer and the Messenger who introduces the players says;

> Messenger;
> Your honour's players, hearing your amendment,
> Are come to play a pleasant comedy;
> For so your doctors hold it very meet,
> Seeing too much sadness hath congealed your blood
> And melancholy is the nurse of frenzy;
> Therefore they thought it good you hear a play
> And frame your mind to mirth and merriment,
> Which bars a thousand harms and lengthens life.
>
> (Introduction)

Here again are illusions to the humoral doctrines of Hippocrates and Galen, as well as good advice for psychiatric treatment.

In Coriolanus is excellent advice both physiological and psychological on how to gain favors;

Sicinius Velutus, a tribune of the people begs Menenius to intercede with Coriolanus that he may spare Rome. Menenius in planning how he should approach the conqueror gives some good psychology and physiology on how to secure favors.

> Menenius; I'll undertake it;
> I think he'll hear me. Yet, to bite his lip
> And hum at good Cominius, much unhearts me.
> He was not taken well; he had not dined;
> The veins unfilled, our blood is cold, and then
> We pout upon the morning, are unapt
> To give or forgive; but when we have stuffed
> These pipes and these conveyances of our blood
> With wine and feeding, we have suppler souls
> Than in our priestlike fasts; therefore I'll watch him
> Till he be dieted to my request,
> And then I'll set upon him.
>
> (Act V, Scene 1)

guests. Prompted by his wife he says;

present crime, that he scarce can welcome or entertain his

In the banquet scene Macbeth is so distraught with past and

in the Bible. They did not escape Shakespeare.

ments expressing modern physiological ideas. Many are found

states to digestion, but long before his day there were state-

It was Pawlow who demonstrated the relation of emotional

> "Now good digestion wait on appetite
> And health on both."
>
> (Act III, Scene 4)

In the Comedy of Errors the author returns to the same thought. Antipholus of Syracuse, mistaken by Adriana for her own husband, the twin brother, Antipholus of Ephesus, has been driven almost if not entirely crazy by Adriana's scoldings. He takes refuge in an abbey and wins the sympathy of the Abbess who reproves Adriana with these words;

> "Thou say'st his meat was sauced with thy upbraidings;
> Unquiet meals make ill digestions."
>
> (Act V, Scene 1)

Shakespeare has many references to the minor ills and inconveniences of the body.

The Comedy of Errors has an interesting passage having to do with baldness.

Antipholus Syracuse says;
"There's a time for all things."
Dromio, his servant, replies,
"There's no time for a man to recover his
hair that grows bald by nature"
Antipholus;
May he not do it by fine and recovery?
Dromio;
Yes to pay a fine for a periwig and recover
the lost hair of another man.
Antipholus;
Why is time such a niggard of hair, being,
as it is, so plentiful an excrement?
Dromio;
Because it is a blessing that he bestows
on beasts; and what he hath scanted men in
hair he hath given them in wit.
Antipholus;
But ther's many a man hath more hair than wit.
Dromio;
Not a man of these but hath the wit to lose his hair.
(Probably referring to the French disease)
(Act II, Scene 2)

In Much Ado About Nothing, Benedict, secretly in love with the beautiful and charming Beatrice is greatly depressed at her feigned indifference. Don Pedro taunts him of his love and Benedict swears he has the toothache.

Don Pedro;
What! sigh for the toothache?
Benedict replies tartly;
Well, every one can master a grief but he that has it.
(Act III, Scene 2)

Shakespeare must have had some toothaches when he wrote this play for later in Act V, Scene 1 he has

Leonata say;
I will be flesh and blood;
For there was never yet philosopher
That could endure the tooth ache patiently.

The Psychiatrists have often claimed that Shakespeare was an honored member of their guild. He does often show a deep insight into human nature, often with a very modern slant.

He was a firm believer in Heredity

Belarius in Cymbeline, says;

> "Cowards father cowards and base things sire base."

Again Suffolk in King Henry the VI, says;

> "For Henry, son unto a conqueror
> Is likely to beget more conquerors."

That hereditary traits may be an excuse for behavior is expressed by Lepidus in Anthony and Cleopatra.

> "His faults in him seem as the spots of heaven—hereditary
> rather than purchased; what he cannot change
> Than when he choose."

Shakespeare several times refers to phobias;

> "Some there are love not a gaping pig;
> Some that are mad if they behold a cat."

Shakespeare seems to have distinguished clearly between idiocy and insanity, a distinction current only in recent years.

In Cymbeline we read,

> "Fools are not mad folk."

Old age and senility are portrayed by Shakespeare with Biblical sympathy and understanding. In "All's Well";

> "For we are old and on our quickest decree,
> The inaudible and noiseless foot of time
> Steals ere we can effect them."

On the other hand he bluntly states,

> "When the age is in, the wit is out."

But he strikes a more cheerful tone in old Adam's sermonette, which I have already quoted;

> "Though I look old, yet am I strong and lusty—."

In

> "Our remedies oft in ourselves do lie
> Which we ascribe to heaven,"

he touches on a bit of medical free will.

But the Freudians would have little to do with the Bard, for he says,

> "Man is but an ass, if he goes about to expound his dreams."

Death Scenes

Two descriptions of death scenes in Shakespeare emphasize what may be called the medical aspects of dissolution and indicate that the author had either made some study of the subject or had been more than usually observing.

The first is Warwick's description of the dead Gloucester in King Henry VI, Part 2.

> "But see, his face is black and full of blood,
> His eyeballs further out than when he lived,
> Staring full ghostly like a strangled man;
> His hair upreared, his nostrils stretched with struggling
> His hands abroad displayed, as one that grasped
> And tugg'd for life and was by strength subdued;
> Look, on the sheets, his hair, you see, is sticking:
> His well proportioned beard made rough and rugged
> Like to the summer's corn by tempest lodged."
>
> (Act III, Scene 2)

In the second part of King Henry the Fourth, the Prince of Wales, Prince Henry, has associated himself with a wild gang of roisterers, of whom Falstaff was chief and the most amusing. On the death of his father, Harry becomes King Henry the Fifth. He banishes Falstaff and all his old misleaders. In the play Henry the Fifth, Falstaff does not appear, but in one scene his death is described by Hostess Quickly. It is a strangely moving scene with a reference to the Hippocratic Fascies and an incident that reminds one of the death of Socrates.

Hostess Quickly;

> "Nay, sure he's not in hell: he's in Arthur's bosom
> if ever man went to Arthur's bosom. A'made a
> finer end, and went away, as if he had been any
> Christian child; . . . a' parted even just
> between twelve and one, even at the turning of the
> tide; for after I saw him fumble with the sheets,
> and play with flowers and smile upon his fingers'
> ends, I knew there was but one way; For his nose

> was as sharp as a pen, and he babbled of green fields. . . . So a' bade me lay more clothes on his feet; I put my hand into the bed and felt them, and they were as cold as any stone: . . .
>
> (Act II, Scene 3)

May I close with two non-medical references which however illustrate the versatility of this great poetic and dramatic genius.

No play runs the gamut of human emotions more than Antony and Cleopatra. Love, fear, hate, remorse all and more are expressed by the poet. Humour, too, is intermingled with the rest.

The three great triumvirates are met on board Pompey's galley.

Anthony has just returned from a little escapade in Egypt, where he was attracted by the wit and beauty of Egypt's queen. Like many a traveler from a far and strange country he is evidently spinning yarns about the wonderful things in Egypt; the Nile, which foretells the future crop by the height to which it rises; the sowing of the seed in the rivers slime and ooze; and the strange serpents bred of the mud by the operation of the sun. And Lepidus, curious, asks,

> "What manner o' thing is this your crocodile?"

And Antony replies,

> "It is shaped, sir, like itself; and it is as broad as it hath breadth; it is just so high as it is, and it moves with its own organs; it lives by that which nourisheth it."
>
> "What color is it of?" Lepidus asks.
>
> "Of its own color too," Antony replies.
>
> 'Tis a strange serepnt," remarks Lepidus, and with a parting shot, Antony agrees,
>
> "'Tis so. And the tears of it are wet."
>
> (Act II, Scene 7)

Shall I draw a moral and say that many of our answers to examination questions as well as some of the questions of life are no more informative than Anthony's humorous description of the crocodile.

Sometimes Shakespeare is most strangely and uncannily modern.

In Timon is this description of the attitude of many a modern politician.

> "Promising is the very air o' the time; it opens the eyes of expectation; performance is ever the duller for his act; and, but in the plainer and simpler kind of people, the deed of saying is quite out of use. To promise is most courtly and fashionable; performance is a kind of will or testament which argues a great sickness in his judgment that makes it.
>
> (Act V, Scene 1)

And what could the poet have been thinking of when in Julius Caesar he had Calpurnia, Caesar's wife say,

> "Fierce fiery warriors fight upon the clouds,
> In ranks and squadrons and right form of war,
>
> (Act II, Scene 2)

And so we leave our great contemporary, William Shakespeare.

CLINICAL EFFECTS OF THE STING OF THE "PUSS CATERPILLAR" (*MEGALOPYGE OPERCULARIS* S. & A.) ON MAN*

DON W. MICKS

As early as 1913 the larval stage of the moth *Megalopyge opercularis*, also known as the "puss caterpillar" which is distributed throughout the southern states, was known to cause dermatitis in south Texas. Especially large numbers of cases were reported during the years 1913 and 1920 (Foot, 1922). According to Bishopp (1923) these caterpillars became so abundant around some of the public schools in San Antonio and the stings were so frequent that the schools were closed until the larvae could be brought under control. Foot (1922) conducted a careful study of the pathology of the dermatitis and found that it was caused by a venom introduced into the skin by the hollow, poisonous spines of the larva.

During the months of October and November, 1951, in Galveston and adjacent areas, "puss caterpillars" (Fig. 1) were present in unusually large numbers and eighteen cases of stings in man were brought to our attention—eight of these suffering symptoms severe enough to require emergency hospital treatment. Inasmuch as the severity of reactions to such stings occurring during this particular season appeared to be markedly greater than reports of previous years would indicate and with dermatitis as one of the less significant findings, it would seem worth-while to revise our impressions as to the potential effects of "puss caterpillar" stings. It is therefore the purpose of this report to present a series of cases with methods of treatment used, in an effort to provide some basis for ready recognition and treatment of the more seriously affected individual.

Review of Cases

A total of 18 patients are included in this series, 8 of whom required emergency treatment. The remaining 10 exhibited

*From the Laboratory of Medical Entomology, Department of Preventive Medicine and Public Health, University of Texas Medical Branch, Galveston, Texas. Received for publication January 20, 1952.

relatively mild symptoms and were not treated. Although practically all age groups from 6 to 63 years of age are represented, with about the same distribution as to sex, neither the severity of the sting nor the symptoms could be correlated with either the age or the sex of the patient. There was, however, a fair degree of correlation between the severity of reaction and the

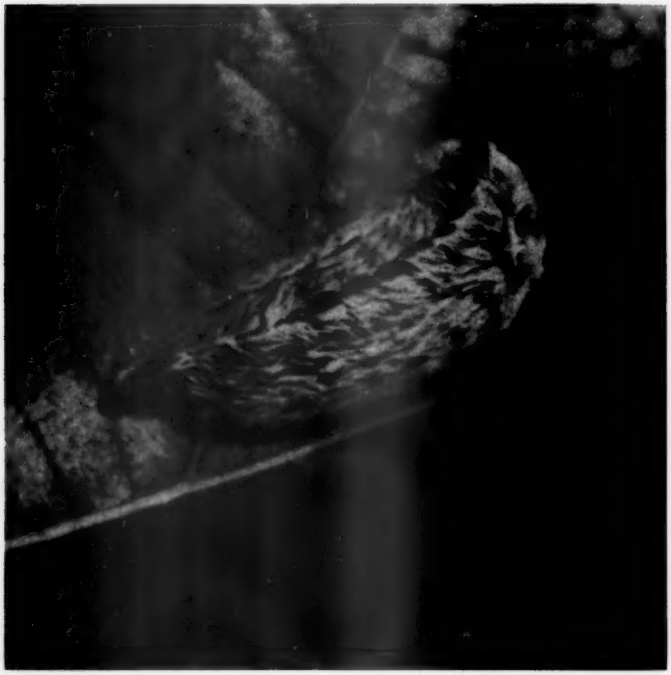

FIG. 1. Mature larva of the "puss caterpillar" measuring 2.2 cm. in length. This stage feeds on the leaves of oak, elm, or sycamore trees.

size of the caterpillar and the amount of pressure upon contact. The hand or arm was the site of the sting in 10 of the patients; the foot or leg was involved in 5; while the neck or face was stung in the remaining three. There was no relationship between the site of the sting and the sequence of constitutional symptoms. Table I summarizes the syndrome in the

entire series. In each instance an intense, burning pain at the site of the sting was immediately experienced, followed in a matter of minutes by an area of erythema of 3 cm. or less in diameter. In several of the patients, small white spots appeared

TABLE I

Frequency of symptoms in 18 cases of "puss caterpillar" sting.

Number of cases	Shooting Pain	Localized Swelling	Nausea and Vomiting	Lymphadenitis	Dermatitis (2-3 days)
18	18	5	4	8	14

in this area of inflammation. The most characteristic symptom was the presence of "shooting pains." When the sting occurred on one of the limbs, the pain extended proximally from the area of the sting to the adjacent axillary or inguinal region. Although nausea, vomiting and localized swelling in the area of the sting occurred in several of the patients, the majority of them did not present these symptoms. Regional lymph nodes were palpable in eight of the individuals examined. The majority of patients exhibited a mild dermatitis which lasted for 48 to 72 hours after the sting, perhaps varying with the amount of venom inoculated. The patient who stepped on a caterpillar (Case 1) had a small area of dermatitis which persisted for about a week.

A total of eight patients were treated with either an oral antihistamine, with calcium gluconate, or with both. Four of these cases are reported in detail.

Report of Cases

Case 1. A 26-year-old white female stepped on a "puss caterpillar" while walking barefoot in the house. There was an immediate and intense burning sensation in the instep of her right foot and within 5 minutes an erythematous area 1.5 cm. in diameter with a white center developed. At this time the patient went into shock exhibiting some respiratory embarrassment. When aroused, she complained of severe pains shooting up the ankle into the leg. Approximately 15 minutes after the sting, the pains had extended to the right inguinal

region. She was then taken to a hospital emergency room where her blood pressure was found to 70/30 with a slow pulse rate. The patient was given 100 mg. of pyribenzamine orally with no appreciable relief within 30 minutes. At this time 10 cc. of a 10% calcium gluconate solution were administered intravenously. Shortly thereafter the patient experienced relief from the acute pain and complained more of a generalized numbness of her right leg and foot. The following day she was able to go to work but still felt some numbness in her right foot. For several days thereafter, there remained at the site of the sting several small areas of erythema with considerable itching.

Case 2. A 9-year-old white male was admitted to the emergency room shortly after having been stung on the dorsum of his right hand by a mature larva. The patient was suffering agonizing local pain which in 10 minutes had extended into the right axilla. There was an area of erythema at the site of the sting approximately 3 cm. in diameter. Following a short episode of nausea and vomiting calcium gluconate was given intravenously. The pain began to subside very shortly thereafter and within 45 minutes the patient was quite comfortable and able to return home. No swelling of the hand was noted although it remained very sensitive to light pressure for several hours after the sting.

Case 3. A 58-year-old white female was admitted with a sting on the palm of the left hand, at which time an area of erythema approximately 2.5 cm. in diameter was noted. The patient complained of sharp, stabbing pains which she first felt in the wrist and lower arm and which soon extended to the left axillary region. She also felt a dull pain in the adjacent thoracic region. Pyribenzamine (100 mgm.) was administered with no apparent relief after 30 minutes. Codeine (½ gr.) was then given by hypodermic and the patient was taken home. Although the sting occurred about 8:30 A.M. on the day the patient was seen, she was still suffering considerable pain eight hours later when she was re-admitted and complained of feeling dizzy and nervous. At this time the patient exhibited a left axillary lymphadenitis. Calcium gluconate (10 cc. of a 10% solution) was administered and she felt considerable relief from pain shortly thereafter. She was given reassurance for her

anxiety and sent home. The patient was well the following day, but mentioned that she felt a slight stinging in her hand and noticed a small red spot which remained through the following day.

Case 4. A 33-year-old female was seen after having taken a towel from the clothesline to dry her face. In so doing a caterpillar was rubbed across the right cheek to the nose. The patient complained of a severe headache, nausea and sharp pains in the nose and around the eyes. Her nose was slightly swollen and erythematous as was the right side of the face. Pyribenzamine (100 mgms.) was given immediately and the patient experienced some relief although her headache persisted the remainder of the day. On the following day she was asymptomatic except for several small patches of erythema and hypersensitivity of the right cheek.

Discussion

It appears from the foregoing brief data that these moth larvae are not only more numerous during certain years, but also that the venom may vary in toxicity from one season to another. Whereas the observations of both Foot (1922) and Bishopp (1923) suggest that the principal effects of the sting are localized pain and dermatitis, the present study reveals in general a decidedly different and more severe clinical picture. Perhaps the outstanding finding is the presence of acute, stabbing pain and the rapidity with which it extends proximally to the adjacent trunk region in a matter of ten minutes or so after the sting. Apparently the venom passes by day of the lymphatics, since eight of the patients exhibited a pronounced enlargement of the regional lymph nodes. Foot (1922) noted that the local cellular reaction to the poison was chiefly lymphocytic. He found the toxic principle to be thermolabile and suggested that it may be combined with protein vehicles or may itself be a protein. On the other hand, Gilmer (1925) expressed the belief that it is probably not a protein but is adsorbed upon some protein molecule or group of molecules. The exact mode of action cannot be determined until such time when methods are available for securing relatively large quantities of the venom from the poisonous spines (Fig. 2).

Contrary to the findings of Bishopp that stings were especially severe among young children, more severe symptoms were noted in adults of the present series with the children becoming asymptomatic in a relatively short time. In order to

FIG. 2. Dorsal surface of "puss caterpillar" with silky, outer hair pushed aside to reveal the clusters of poisonous spines (A) (photographs by F. W. Schmidt, Department of Medical Photography).

determine the significance of prior sensitization among the older patients, the treated group was questioned for evidence of exposure to this or a related toxin. It was found that only one of the treated adults had been previously stung, presumably by a bee. One nine-year-old white male (Case 2) was stung by a wasp approximately six weeks before his present episode, yet his symptoms were relatively mild compared to the adult group. Furthermore, it is noteworthy that the stings of other venomous arthropods produce an entirely different clinical picture than that described in the present report (Herms, 1950).

The eight individuals who were treated received either an antihistamine (usually 100 mgms.), or calcium gluconate, or both. Although pyribenzamine given alone appeared to alleviate the pain in three of these patients, especially in the case of a sting on the face, it was found necessary to follow this with calcium gluconate. When the latter was used alone, it produced relief from acute pain within a short space of time. In the absence of treatment, symptoms persisted from 6 hours to two days. Since the patients in this series did not respond clinically to antihistamine treatment but did respond to such a non-specific agent as calcium gluconate, this syndrome might be interpreted as an intoxication rather than a histamine-like reaction.

Summary

Eighteen cases of "puss caterpillar" sting in man are reported, eight of whom suffered severe reactions. The symptomatology and response to treatment are discussed.

REFERENCES

Bishopp, F. C.: The puss caterpillar and the effects of its sting on man. *U.S.D.A. Circ.* 288, 14 pp., 1923.

Foot, N. C.: Pathology of the dermatitis caused by *Megalopyge opercularis*, a Texas caterpillar. *J. Exp. Med.*, 35:737–753, 1922.

Gilmer, P. M.: A comparative study of the poison apparatus of certain lepidopterous larvae. *Ann. Entomol. Soc. Amer.*, 18:203–239, 1925.

Herms, W. B.: *Medical Entomology*, Macmillan Company, New York, pp. 563–610, 1950.

EFFECT OF AMPHETAMINE* SULFATE ON VOLUNTARY CHOICE OF ALCOHOL IN ALBINO RATS†

WILLIAM T. MOORE, BERNICE M. MOORE, JOE B. NASH‡ AND G. A. EMERSON

Amphetamine has been used in the therapy of chronic alcoholism in man (Bloomberg, 1939, 1942; Miller, 1942), although results with this agent alone are not uniformly satisfactory (Rosenbaum and Lams, 1942). Krantz and Carr (1951) note that *d*-amphetamine is the "drug which is most generally used in chronic alcoholism," but note that it is useful only in the depressive type of alcoholism. Sollmann (1948) indicates the value of amphetamine as an adjuvant in appropriate instances of chronic alcoholism in man.

Our interest in testing experimentally the effects of amphetamine on voluntary intake of alcohol in rats was stimulated by studies on effects of the amphetamine isomers on maze-running ability in rats (Ewing *et al.*, 1952) and of effects of stress of various sorts on preference for alcohol in rats (Moore *et al.*, 1952). As part of a program of study of the relation between other forms of behavior and increased preference for alcohol in rats, it seemed warranted to test effects of an agent which would affect central nervous system functions to the extent shown by amphetamine.

Experimental

Eighteen female albino rats of the Sprague-Dawley strain, weighing 214–260 g., were placed in individual cages for 35 days. During this period, they were supplied with two drinking bottles, one containing water and the other 5% V/V alcohol in water. The positions of these bottles were interchanged daily,

*"Benzedrine sulfate," racemic amphetamine sulfate, Smith, Kline and French Laboratories, Inc., Philadelphia.

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‡Fellow of the American Foundation for Pharmaceutical Education.

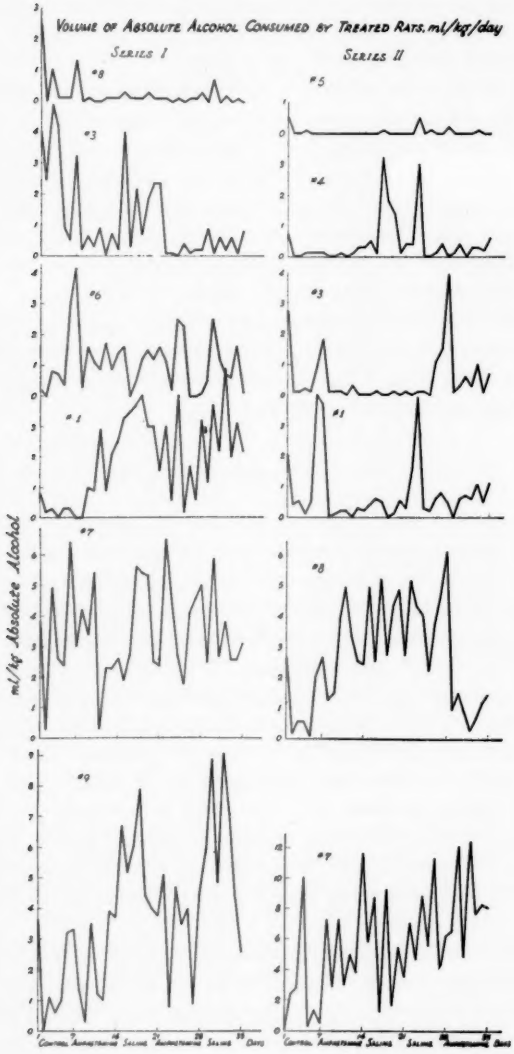
following the technic of Williams *et al.* (1950) as used by us in a former study (Moore *et al.*, 1952). The rats were fed a dog chow which permitted optimal growth.

Nine of the rats, after 7 days without treatment, were given 4 mg./kg. of amphetamine sulfate subcutaneously as a 0.4% solution in saline daily for 7 days, then 1 ml./kg. of 0.9% NaCl subcutaneously for 7 days; the course of amphetamine was then repeated for 7 days, followed by a 7-day course of saline injections. The other 9 rats were treated similarly except that 2 courses of saline injections separated the 1st and 2d amphetamine courses; the control period was followed by a course of amphetamine for 7 days, then 14 days of saline injections, ending with a final course of amphetamine injections. This was done to avoid any disturbing environmental factors, since the rats served as their own controls.

Results

Seven of the rats treated as noted above showed a decrease in average daily alcohol consumption during both of the periods during which amphetamine was given. Nine showed a decrease during either the 1st or 2d course of amphetamine. Only 2 rats showed an increase in alcohol consumption during both periods of treatment with amphetamine; in the one of these in which the 2d course of amphetamine was followed by 7 days of saline injections, alcohol consumption increased further during this final course of saline injections. Since our previous work (Moore *et al.*, 1952), we have come to agree with Williams *et al.* (1950) that prolonged periods of observation are necessary to define fully the behavior of rats in regard to alcohol choice; results of short-term studies must be interpreted carefully since certain rats show a tendency to increase their intake of alcohol with time.

Figure 1 illustrates the responses of 12 rats to treatment with amphetamine. Rats 3, 6 and 8 in series I, and rats 1, 3, 4 and 5 in series II show a decreased intake of alcohol during both courses of amphetamine. Rats 1 and 9 in series I, and rat 8 in series II show a decrease only during the 2d course of amphetamine, while rat 7 of series I shows a decrease only during the


Volume of Absolute Alcohol Consumed by Treated Rats. ml/kg/day
Series I
Series II
#8
#5
#3
#4
#6
#3
#1
#1
#7
#8
#9
#7
ml/kg Absolute Alcohol
Control
Anesthesia
Saline
Anesthesia
Saline
Anesthesia
Saline
Days

1st course of amphetamine. Rat 7 of series II, like rat 5 of series I which is not illustrated, shows a regular increase in alcohol intake throughout the experimental period, despite treatment, except for the 7 days immediately following the 1st course of amphetamine.

Despite the clear indications of an effect in certain rats, the total alcohol and water consumptions for the 2 groups over each of the experimental periods do not reflect significant differences in preference for alcohol in either group. Total fluid intake increased some 25–30% in both groups, probably due to increase in the ambient mean temperature from the middle of June until the end of July, in Galveston, while changes in alcohol consumption were of much greater magnitude. Preference for alcohol is not truly expressed by calculations from total alcohol intake for the groups, since both of the rats which increased their intake during amphetamine therapy had, by far, the highest initial alcohol intakes, in each group. If calculations are made from averages of daily preferences of individual rats for alcohol, the results more nearly conform to those expected from Figure 1, but the short duration of the control period causes the reference value to be artificially low. Similarly, if the volume of alcohol consumed by each individual rat is calculated for each of the experimental periods in terms of % of the initial average daily volume consumed by each rat during the control period, the results are artificially high due to the misleading low values obtained in a brief control period. Even so, if results for rat 1 of series I are omitted, the average % of the initial control daily volume of alcohol consumed by the remaining 17 rats, so calculated for the period of the 1st course of amphetamine, is 74.3%.

In our previous study (Moore *et al.*, 1952), we noted that preference for alcohol, expressed as % of 5% alcohol consumed in terms of total fluid intake, was of somewhat greater significance than the actual volume of alcohol intake, for any behavioristic conclusions. In addition, the % change in volume consumed in reference to the average daily volume of intake during an adequately prolonged control period should yield a criterion which would permit statistical evaluation. Williams (1951) notes the difficulty of statistical examination of results in

any studies on alcoholism; we believe that a properly designed experiment with laboratory animals, with a suitable control period for stabilization of alcohol preference, is amenable to statistical treatment if the above criteria of change are considered in addition to the actual changes in volume of alcohol consumed. In this belief, we have no difference of opinion on the matter that the human chronic alcoholic is an individual problem and must be treated in each instance as such.

Discussion

Bloomberg (1939) reported successful results with amphetamine therapy of chronic alcoholism in 14 of 21 cases, with 4 failures; his 2d (1942) publication noted good results in 7 of 12 patients, with 1 failure. Miller (1942) found that 397 of 487 ambulatory alcoholic patients treated with amphetamine had remained abstinent when examined after 4–14 months. Rosenbaum and Lams (1942) had good results in only 3 of 24 patients, but report an additional patient who relapsed after 10 months of abstinence; they question whether similar results might not be obtained with a placebo, discuss the importance of psychotherapy, and note that patients who pay for treatment may have better results than those to whom treatment is given gratis. All these latter factors may be avoided in animal experiments. Against favorable results in 67, 58, 82 and 17%, respectively, of patients in the series noted above, comparable results were obtained in 39% of rats treated under the conditions of the present study. Failures of amphetamine therapy in man, reported as 19 and 8% in Bloomberg's (1939, 1942) series, are comparable to the 11% failures in our series of rats.

Mechanism of action of amphetamine in affecting chronic alcoholism in man is usually considered (Bloomberg, 1939; Rosenbaum and Lams, 1942) as a form of substitution therapy. The latter workers note that certain anxieties due to difficulties in interpersonal relations may be relieved by the euphoria or sense of security attendant on the use of amphetamine. As discussed by Moore *et al.* (1952), energy bound by anxiety may be liberated by effects of alcohol; amphetamine may act similarly, relieving the urge for alcohol. Biochemically, some anal-

ogy may be drawn between the action of amphetamine in reducing the concentration of aldehydes in the central nervous system by competitive affinity for amine oxidase and a phase of the supposed metabolic pathway of ethanol, which by mass action effects may reduce the concentration of aldehydes more toxic than acetaldehyde. Such speculations must face the reality of the statement by Rosenbaum and Lams (1942) that "Inasmuch as there is no specific type of personality involved in alcoholism, and no specific type of mental conflict as the underlying basis for drinking, any approach to the problem could not be limited to the administration of one drug but would need be more catholic." As these authors point out, one alcoholic may be insecure, while another uses drinking as an hostile act. No simple mechanism of action may be hypothesized in this complex situation, unless it is assumed that all alcoholics feel a significant degree of anxiety, sufficient to bind considerable energy. In any case, the present results do not disagree with the consensus that amphetamine, or preferably its dextro isomer, is most useful in therapy of chronic alcoholism in man to allow time for institution of effective psychotherapeutic measures.

Hulpieu and Cole (1946) note that 0.05 mg./kg. of epinephrine, intravenously, greatly increase the initial concentration of alcohol in the brain and blood of animals following an immediately subsequent dose of alcohol, while the initial concentration of alcohol in the muscles is greatly reduced. This difference in distribution of alcohol in animals treated with epinephrine is sufficient to explain the considerable potentiation of depressant effects, without recourse to a purported auxoneurotropic action of epinephrine. While the latter effect might be shown by amphetamine, the weak pressor effect of this agent would suggest that no comparable change in distribution of alcohol would occur in animals treated with amphetamine instead of epinephrine. It is unlikely that the decreased alcohol intake in rats while receiving amphetamine is due to an increased concentration and effect of alcohol in the central nervous system.

With the present small series, further handicapped by the brief control period which did not permit recognition of latent high preference for alcohol in some of the rats, conclusions may

be fallacious. A suggestive observation, however, is that 6 of the 7 "successes" in reducing alcohol intake during both courses of amphetamine occurred in rats with a low preference for alcohol, with an average intake of only 0.08–1.9 ml./kg./day of absolute alcohol. In those with moderate preference for alcohol, with a control average intake of 2.5–3.5 ml./kg./day of absolute alcohol, only rat 3 of series I showed a markedly decreased alcohol intake during both periods of amphetamine therapy, while rat 5 of series I and rat 7 of series II both showed an increase in alcohol intake over the entire 35 days despite therapy. Rat 7 of series I, and rat 9 of series I which was not initially "alcoholic," showed a decreased alcohol intake during only 1 of the 2 courses of amphetamine. The observation that 1 rat with an initially moderate alcohol intake, corresponding to nearly a pint of 100 proof spirits/day for man, was affected by amphetamine therapy to reduce her alcohol consumption to an equivalent for man of less than 1½ fluid ounces of 100 proof spirits/day during both courses of amphetamine therapy is of some interest. This indication that amphetamine may act on alcoholics and those who use alcohol sparingly alike possibly denotes a fundamental mechanism of reducing the urge to drink. The "failures" may be explained most facilely by taking into consideration that certain rats may have genetotrophic factors which cannot be overcome by moderate therapy; *i.e.*, that there may be a threshold for this action of amphetamine.

A necessary limiting factor in drawing analogies between results of the present study and results of therapy in man is that the dose of amphetamine, admittedly high as used here, was administered subcutaneously to the rats but once a day, while the accepted clinical therapy in man requires at least 2 oral doses a day. Duration of action has a vital rôle in affecting the whole psychosomatic reaction, despite the difference in species susceptibility to central nervous system effects of amphetamine.

Summary

Seven of 18 rats treated subcutaneously with 4 mg./kg./day of amphetamine sulfate for 2 non-consecutive 7-day periods during 35 days showed a decreased intake of alcohol during both

courses, when given a choice of drinking 5% V/V alcohol or water. Nine rats showed a decrease during the 1st or 2d course of amphetamine therapy for 7 days. The mechanism of action of amphetamine in human chronic alcoholism, the difficulties of statistical evaluation of results of human and animal experimentation in this field, and some suggestions as to means of ameliorating these difficulties in animal experiments are discussed.

Acknowledgment

We are grateful to Smith, Kline and French Laboratories, Philadelphia, for supplying us with the amphetamine sulfate used, and for prior support of basic studies helpful to the present work.

REFERENCES

Bloomberg, W.: Treatment of chronic alcoholism with amphetamine (benzedrine) sulfate. *New England J. Med.*, 220:129–135, 1939.

Bloomberg, W.: Results in the use of amphetamine (benzedrine) sulfate as an adjuvant in the treatment of chronic alcoholism. *Am. J. Psychiatry*, 98:562–566, 1942.

Ewing, P. L., Bernice M. Moore and W. T. Moore: The effect of amphetamine and related compounds on maze performance of white rats. *J. Pharmacol. and Exptl. Therap.*, in press.

Hulpieu, H. R. and V. V. Cole: Potentiation of the depressant action of alcohol by adrenalin. *Quart. J. Studies on Alcohol*, 7:89–97, 1946.

Krantz, J. C., Jr., and C. J. Carr: Pharmacologic Principles of Medical Practice. Williams and Wilkins Company, Baltimore, 2d ed., 1951.

Miller, M. M.: Ambulatory treatment of chronic alcoholism: a clinical study. *J. Am. Med. Assoc.*, 120:271–275, 1942.

Moore, W. T., B. M. Moore, Joe B. Nash and G. A. Emerson: Effects of maze running and sonic stimulation on voluntary alcohol intake of albino rates. *Texas Repts. Biol. and Med.*, 10: 59–65, 1952.

Rosenbaum, M. and L. Lams: Use of amphetamine (benzedrine) sulphate in the treatment of chronic alcoholism. *Am. J. Psychiatry*, 9:680–683, 1942.

Sollmann, T.: A Manual of Pharmacology and its Applications to Therapeutics and Toxicology, 7th ed., W. B. Saunders Co., Philadelphia, 1948.

Williams, R. J., L. J. Berry and E. Beerstecher: Genetotrophic diseases: alcoholism. *Texas Repts. Biol. and Med.*, 8:238–256, 1950.

Williams R. J.: Nutrition and Alcoholism. U. Oklahoma Press, Norman, 1951.

AN INEXPENSIVE AND SIMPLE REFRIGERATING MANTLE FOR ANGLE CENTRIFUGES*

WIKTOR W. NOWINSKI AND WASLEY D. YUSHOK

The present biochemical techniques, particularly those concerned with purification of enzymes and isolation of single cell components, in majority of cases, require centrifuging in a refrigerated unit. In laboratories which do not have a cold room available, refrigerated centrifuges have to be used. However, the latter are generally expensive and occupy a large amount of space. In our work on the desoxyribose nucleic acid estimations, it was necessary to centrifuge our material in the cold. Therefore, we devised a simple refrigeration mantle for a Precision "Centricone" Micro-centrifuge and which placed over the centrifuge, maintained the desired range of temperature in the tubes for any length of time.[1] In our tests, during 15 minutes of centrifugation with full load at the speed of 1900 r.p.m., the initial temperature of 4° C. of the tube contents was not altered.

Description of the Refrigeration Mantle

The refrigeration unit consists of the mantle (Fig. 1, middle), a top tray (Fig. 1, right), and cork insulated lid (Fig. 1, left). It is made of monel metal; however, other suitable non-rusting metals can be used. The Fig. 1 also shows the very close fit of the mantle over the centrifuge. The air space between the centrifuge shield and the mantle should not exceed ½ inch.

Figure 2 shows the details of construction. The mantle consists of two cencentric compartments: the inner of 1¼" width is open at the top and serves as a container for the cooling agent. It has a faucet attached on bottom in order to drain the water, if ice is used. The outer compartment of 1" width is packed with

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[1]The mantle was built by Mr. A. P. Freund, Technical Apparatus Shop, University of Texas Medical Branch. We wish to thank Mr. Freund for his help and suggestions.

cork for insulation and is completely enclosed. To the bottom of the mantle, three legs, 3½″ high, are fixed. The other details are selfexplanatory.

For the convenience of filling and to avoid pieces of ice or dry ice from falling into the centrifuge, a flat cover, of a

FIG. 1. Refrigeration mantle placed over the centrifuge. Left: cover, insulated with cork; right: ice tray to be put on the top of inner compartment.

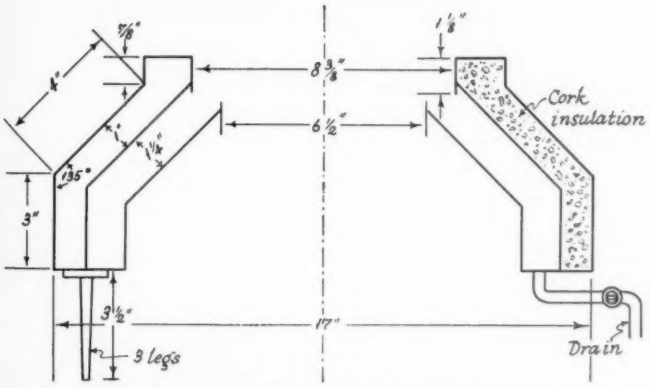

FIG. 2. Diagrammatic cross section of the refrigeration mantle.

diameter of 6¾″ (not shown in either figure) is put over opening above the centrifuge. After filling the inner compartment, the flat cover is removed. The ice tray (Fig. 1, right), which also contains the cooling agent, is then placed on the mantle, and the entire system is closed with the cork insulated cover.

Generally, we pre-chill the centrifuge about 30 minutes before it is used. A wide range of cooling agents can be used; the proper amount has to be found out empirically in order to obtain the desired temperature. When excessive amounts of dry ice are used, the samples freeze during centrifugation.

The centrifuge also can be used at higher temperatures than that of room, for instance, for the elution of antibody from erythrocytes (45° to 55° C.).

Adaptation of the basic design to other types of angle centrifuges, requires only corresponding changes in dimensions.

Summary

A simple, inexpensive mantle, for various types of angle centrifuge is described. It maintains the desired temperature for any amount of time necessary for centrifugation.

PROCEEDINGS OF THE TEXAS BRANCH OF THE SOCIETY OF AMERICAN BACTERIOLOGISTS

Fall (10th Anniversary) Meeting, The University of Texas, Austin, Texas

December 7–8, 1951

Titles

Strain variation of *Salmonella* in relation to resistance to Streptomycin. R. A. Finkelstein and C. E. Lankford, Department of Bacteriology, The University of Texas, Austin, Texas.

The *in vitro* effect of Neomycin on bacteria isolated from cutaneous pyogenic infections. C. S. Livingood, C. Elizabeth Spoor, Rose Marie Sautter, and Frank B. Engley, Jr., Department of Dermatology and Syphiology, and Department of Bacteriology, The University of Texas Medical Branch, Galveston, Texas.

Morphological studies of some filamentous microorganisms observed in material obtained from teeth. W. A. Nolte, Department of Microbiology, The University of Texas School of Dentistry, Houston, Texas.

The History of Bacteriology in Texas. O. B. Williams, Department of Bacteriology, The University of Texas, Austin. (By request of the Texas Branch, S.A.B.)

The apparent catalytic effect of methionine on the utilization of homocystine by brucellae. L. J. Rode, Chas. E. Lankford and V. T. Schuhardt, The Brucellosis Research Project of the Clayton Foundation and the Department of Bacteriology, The University of Texas, Austin.

The prevalence of *Coxiella burnetii* in Central and South Texas dairies. Margaret Ann Norris, Mary Horan and J. V. Irons, The Texas State Department of Health Laboratories, Austin, Texas.

Field and laboratory studies on the Coxsackie viruses. Thelma D. Sullivan, Margaret Ann Norris and J. V. Irons, Texas State Health Department Laboratories, Austin, Texas.

The possible role of the cockroach in the dissemination of Lansing poliomyelitis virus. Chin Min Hsiang, Morris Pollard and Don Micks, Department of Preventive Medicine and Public Health, The University of Texas Medical Branch, Galveston.

Bartonella bovis in Texas. Morris Pollard and D. G. Parmer, Department of Preventive Medicine and Public Health, The University of Texas Medical Branch, Galveston, Texas.

I. M. Lewis, *Lecturer*
Dr. J. Werner Braun
Chemical Corps Biological Laboratories
Camp Detrick, Frederick, Maryland
Lecture Topic: *The Role of Amino Acids in Bacterial Population Changes*

ABSTRACTS

In vitro Effects of Some Sulfonamide-substituted Naphthoquinones and Related Agents on *Candida albicans*. Randolph Clements, Joe B. Nash[1] and G. A. Emerson, Department of Pharmacology and Toxicology, The University of Texas Medical Branch, Galveston.

Beck and Lacy[2] reported the high activity of menadione Na bisulfite and 2 other naphthoquinones vs. *C. albicans*, which we[3] confirmed for menadione. Na sulfadiazine is inactive[2] in a concentration of 0.01%, as are several other sulfonamides,[3] including those used in treating enteric infections. Since we had at our disposal ten 1-hydroxy-4-amino-naphthyl compounds diazotized with sulfanilamide, through the generosity of Dr. Roger J. Williams, we tested these compounds in nutrient broth cultures of *C. albicans* by the technique previously used.[3] All were inactive at 0.01%, or at saturation in the medium at pH 6.8. Two of these agents, both containing the 2-methyl group, are effective in treating hypoprothrombinemia in chicks.[4] Mechanisms of fungicidal and hepatotonic effects of menadione and these agents must differ since only a slight per cent of conversion to 1,4-naphthoquinones should result in strong fungicidal activity. Relations of these compounds to other naphthoquinones, halogenated hydroxyquinolines, hydrocupreine esters and triphenylmethane dyes are noted.

The Locus of Action of Streptomycin on the Development of Certain *Clostridium* Species. E. Staten Wynne, Robert E. Collier, and Donald A. Mehl. The Division of Experimental Pathology, The University of Texas M. D. Anderson Hospital for Cancer Research, Houston, Texas, and Department of Plant Sciences, University of Oklahoma, Norman, Oklahoma.

Our thanks are due Professor B. Sharp for offering us facilities for this work.

[1]Fellow of the American Foundation for Pharmaceutical Education.

[2]Beck, O. and H. Lacy: *Texas Repts. Biol. and Med.*, 9:395, 1951.

[3]Howard, J. K., G. L. Jarvis, R. Clements and G. A. Emerson; *Ibid.*, 9:780, 1951.

[4]Ewing, P. L., C. D. Leake, G. A. Emerson and E. Ju-Hwa Chu: *Fed. Proc.*, 4:118, 1945.

In preliminary experiments 1,000 micrograms of streptomycin per ml. prevented the appearance of turbidity in fluid thioglycollate broth inoculated with approximately 500 spores per ml. of *Clostridium perfringens*, *Clostridium chauvei*, or putrefactive anaerobe 3679. This effect could have been due to (1) an inhibition of germination, (2) a sporicidal effect, (3) an inhibition of vegetative cell development, or (4) a combination of these factors.

By the general method of Wynne and Harrell (Antibiotics and Chemotherapy 1:198–202, 1951), it was shown that 1,000 micrograms of streptomycin per ml. did not effect germination. Similarly, a sporicidal action could not be demonstrated. That the site of action of streptomycin in these species was on the vegetative cells was indicated by (1) prevention of turbidity from spore-free inocula, and (2) a lessening of countable colonies with increasing amounts of streptomycin in the counting medium.

Logarithm of the concentration of streptomycin versus logarithm of the number of colonies yielded a straight line over ranges of approximately 25 to 300 micrograms per tube for putrefactive anaerobe 3679 and 6 to 100 micrograms for *C. perfringens*.

An apparent adsorption of streptomycin to spores in distilled water was blocked by the addition of electrolytes.

Studies on a Thermophilic Variant of Bacillus Globigii. Roy B. Mefferd, Jr., and L. Leon Campbell, Jr. Foundation of Applied Research, San Antonio, Texas, and The University of Texas, Austin, Texas.

A variant of *Bacillus globigii* capable of growth at 55°C was isolated by means of subculturing large inocula at increasing temperature increments of 2°C. Streptomycin-resistant mutation incidence was found to increase approximately two-fold for each 10°C increase in the incubation temperature through 37 and 45°C for the mesophilic and thermophilic strains, respectively. Mesophilic *B. globigii* cells in which protein synthesis is blocked, e.g., starvation or dinitrophenol (10–5M) inhibition, lose viability at an increasing rate when incubated at 24, 37, 45 and 55, but not at 4°C. The thermophilic strain,

however, does not lose viability at any of these temperatures. The proteins of the mesophile, being heat labile, are denatured, and since replacement proteins cannot be synthesized the cells become nonviable. The thermophile, having heat stable proteins, is unaffected. Nucleoproteins extracted from each strain and tested for heat stability are found to have very similar properties. The nucleic acids seem to stabilize the protein moiety and prevent detectable denaturation. Results indicate that the protein may actually be denatured, since dissociation of the nucleic acid after heat treatment is followed by detectable denaturation. The nature of the thermophilic resistance of this strain differs from that of the strain of Davis and Williams (1948) producing heat-resistant spores. The latter strain resembles the parent in both growth characteristics and in reaction to ultraviolet radiation. The thermophilic strain is much more stable to the mutational and lethal effects of ultraviolet radiation over a wide temperature range than is either of the mesophilic strains.

A Modified Glass Filter Apparatus for Small Volumes. Frank B. Engley, Jr., Department of Bacteriology, The University of Texas Medical Branch, Galveston.

The UF (ultra-fine) Pyrex filter for bacteriological procedures originally described by Morton (J. Bact. *47* (4), 379, 1944) has been modified for more practical use with small volumes. Standard taper (ST 29/26) tubes have been sealed off at desired lengths to receive small quantities of fluid through the already available 40 mm. filter mantle. It has also been found practical to seal smaller filters (10, 20, 30 mm. sizes) onto mantles employing the ST 29/26 standard taper. An all glass chemically clean sterilizing filter for small volumes has thus been made available which has the advantages of economy in original cost, smallness in size, ease of handling and recovery of greater amounts of the original fluid following filtration. The filter apparatus has found usefulness for the filter-sterilization of growth-factors, amino acids, rare sugars, sera, drug preparations and antibiotics where small volumes of heat labile fluids require sterilization.

The Effect of Cortisone on Coxsackie Virus Infections in Adult Mice. S. Edward Sulkin, H. Craig Wallis and Paul Donaldson. Southwestern Medical School of The University of Texas, Dallas.

When cortisone was used as an adjuvant, 15 gram adult mice could be fatally infected with certain strains of Coxsackie viruses. Moreover, serial passage of these agents could be maintained with the aid of cortisone in such mice, thus confirming the observations reported by Kilbourne and Horsfall (Proc. Soc. Exp. Biol, and Med. 77:135, 1951).

The Group B Conn. 5 strain was propagated in this manner for five serial passages. Occasional deaths followed intranasal instillation of the virus in both 7 and 15 gram cortisone-treated mice. The Group B Nancy strain was likewise maintained for five serial passages. More than 85 per cent of all 15 gram mice treated with 5 mg. of cortisone were fatally infected following intraperitoneal injection of both viral strains. However, with the intranasal route of inoculation 90 per cent mortality occurred in both 7 and 15 gram cortisone-treated and control mice. In contrast to the strains mentioned, the Group B Ohio R strain did not produce overt infection following intraperitoneal injection of either 7 or 15 gram mice treated with cortisone, but intranasal administration of the virus was regularly fatal in cortisone-treated 7 gram mice. The Dalldorf types, 1, 2, and 3 as well as the Dallas-M.B. strains of Group A Coxsackie viruses failed to infect 15 gram cortisone-treated mice following intraperitoneal injection.

Effect of Sanguinin, an Antibacterial Agent from Blood, on Streptococcal Infection in Mice. Don W. Micks, Dorothy M. Whitney and Ludwik Anigstein. Department of Preventive Medicine and Public Health, The University of Texas Medical Branch, Galveston

The blood hydrolysate Sanguinin was tested *in vivo* in 268 albino mice accompanied by 221 untreated controls for its effectiveness against the betahemolytic *Streptococcus zooepidemicus*. Sanguinin solution (20 mg./ml.) was inoculated subcutaneously (0.25 to 1.0 mg./mouse daily, usually for 4–6

days) either prior to or after infection. Suppressive effect on the course of the infection was noted in all series of treated mice. A significant increase in survival time was noted in the pretreated series with an average survival percentage of 38.9 compared with 6.0 of the total of untreated controls. It does not appear that a direct correlation can be established between the survival rates and the intake of Sanguinin or the length of its administration; however, pretreatment seems to be of decisive value.

Studies on So-Called "Direct" Antagonism in Bacteria. James O. Norman and E. Staten Wynne. Division of Experimental Pathology, The University of Texas M. D. Anderson Hospital for Cancer Research, Houston, Texas.

In studies with 4 *Aerobacter* strains and 6 *Gaffkya* strains, the production of heat-labile and chloroform-labile antibiotic substances capable of diffusion through agar has been demonstrated for all strains by two techniques.

Thin radial sectors of nutrient agar were placed on nutrient agar plates "staled" with the antagonist, and test species streaked after preliminary incubation were completely inhibited in all cases. By the second method, plates of phenylethyl alcohol medium were prepared with "center wells" filled with "staled" nutrient agar. Test species streaked after 2 weeks' incubation were completely inhibited in nearly all cases.

With both methods, plates showing inhibition were exposed to chloroform vapors and re-streaked with test species. Growth in all cases was comparable to that on controls.

Test species used with the *Aerobacter* strains were *Aerobacter aerogenes* 8308, *Salmonella paratyphi*, *Bacillus subtilis*, *Bacillus polymyza*, *Bacillus anthracis*, *Bacillus mycoides*, *Shigella paradysenteriae*, and *Micrococcus pyogenes* var. *aureus*. With *Gaffkya* strains, test species were *Bacillus polymyxa*, *Microcaccus pyogenes* var. *aureus*, *Salmonella paratyphi*, *Shigella sonnei*, *Shigella paradysenteriae*, and *Gaffkya tetragena* 6007b.

Only the Gram-positive species were used with the "center well" technique.

Serological Studies with *Vibrio Metschnikovii*. Cora L. Gott, E. Staten Wynne, Donald A. Mehl, and James O. Norman. Division of Experimental Pathology, The University of Texas M. D. Anderson Hospital for Cancer Research, Houston, Texas.

In studies of immune response of humans to a *Vibrio metschnikovii* vaccine, neither vibriocidal effects nor saline agglutination at 56°C, with living or heat-killed antigen, could be demonstrated. However, agglutinated growth occurring in broth containing immune serum could be used as a quantitative measure of response. The antibody involved could be removed by absorption with living or dead antigens.

Developing tests were positive with living antigen, but not with heat-killed antigen. A fragile agglutination could be obtained by incubation at 37°C (phase I of the Coombs test), followed by centrifugation. Addition of anti-human globulin caused a more stable agglutination.

Absorption with heat-killed antigen or a supernatant from centrifuged heat-killed antigen resulted in removal of the antibody responsible for growth agglutination and phase I Coombs agglutination, but the developing test remained positive. Absorption with living antigen removed both antibodies. Results with phenol-killed antigens paralleled those with living antigens.

The antigenic structure of *V. metschnikovii* is postulated to consist of (1) a heat-stable surface antigen, possibly flagellar, and easily separated from the cell; and (2) a heat-labile antigen. The first is demonstrated by growth agglutination and the second by the developing test.

Alcaligenes Faecalis Meningitis and Bacteremia Concurrent with Bacillary Dysentery. E. Staten Wynne, Jacob W. Old, and Cora L. Gott. Department of Pathology, The University of Texas M. D. Anderson Hospital for Cancer Research, Houston, Texas.

Only 7 cases of meningitis bacteriologically proven due to *Alcaligenes faecalis* have been reported thus far.

The present case is believed to be the first occurring concurrently with bacillary dysentery. *Shigella paradysenteriae* type II and *A. faecalis* were isolated from contents of the colon. The latter organism was also obtained from blood and cerebrospinal fluid.

Pathological findings in the terminal ileum, colon, and meninges were consistent with the view that the bacillary dysentery was a predisposing factor for invasion of the blood stream and meninges by the ordinarily non-pathogenic *A. faecalis.*

Gamma-aminobutyric Acid in Certain Fungi. Robert Fuerst and Jorge Awapara. Department of Biochemistry, The University of Texas M. D. Anderson Hospital for Cancer Research, Houston.

Gamma-aminobutyric acid has been demonstrated to be a normal constituent of the free intracellular amino acid fraction of *Neurospora crassa Emerson wild 5256A, Neurospora crassa Stanford Univ. Strain 1, wild type, sex A, Neurospora crassa 47305A, Pro.Glu.-less mutant, Neurospora, Non-conidial W541-8a, Neurospora, albino 15300 R3a, Aspergillus niger,-Strain 215-4247, Steinberg,* and *Penicillium notatum, Strain 1249.B21.* The compound exhibited growth stimulating properties for all *Neurospora* strains tested, and could be utilized as a nitrogen source. Although the metabolic pathway of gamma-aminobutyric acid in *Neurospora* was not established unequivocally by this investigation, evidence has been presented that gamma-aminobutyric acid may be metabolized by transamination with alpha-ketoglutaric acid.

MORPHOLOGICAL OBSERVATIONS BY ELECTRON MICROSCOPY OF THE LANSING STRAIN OF POLIOMYELITIS VIRUS AFTER PROPAGATION IN THE SWISS ALBINO MOUSE*

REGINALD L. REAGAN AND A. L. BRUECKNER

The Lansing strain (Armstrong, 1939) of poliomyelitis virus used for this study was furnished by Dr. David Bodian of the Poliomyelitis Research Center, Johns Hopkins University, Baltimore, Maryland. At that laboratory it was passed 200 times in Swiss albino mice by the intracerebral route of inoculation, and at this laboratory it was passed 6 additional times before initiating the following experiment.

Four Swiss albino mice (3-weeks-old) were inoculated intracerebrally with 0.03 ml. of a 10% dilution of poliomyelitis virus from the 206th intracerebral mouse passage. The mice showed typical symptoms of poliomyelitis on the 5th and 6th days. When symptoms appeared the mice were sacrificed; the brains and cords were removed aseptically and stored in a solid carbon dioxide chest at —40°C. Four mice were injected intracerebrally with a 10% suspension of normal mouse brains. No symptoms of poliomyelitis were noted in these normal controls. These 4 mice were sacrificed on the 5th day post inoculation. The brains and cords were removed aseptically and stored under the same conditions as above.

Four days later the pools of infected mouse brain and cord and of normal mouse brain and cord were removed from storage, thawed at room temperature, ground with alundum, and diluted to 10% suspensions with physiological saline. The 2 suspensions were then subjected to 3 minutes centrifugation at 1,000 rpm in an angle centrifuge. The supernates were removed from the specimens and filtered through type ST size L3 Seitz filters. The filtrates were then subjected to centrifugation in a Spinco ultracentrifuge for 3 hours at 50,000 rpm under refrigeration.

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*From the Live Stock Sanitary Service Laboratory, Maryland State Board of Agriculture, University of Maryland, College Park. Received for publication December 30, 1951.

The temperature of the refrigerated outer jacket stayed constant at —10°C during the centrifugation. After the 3-hour centrifugation period, the supernates from the specimens were discarded and the sediment from each specimen was resuspended in 1 ml. of physiological saline.

A small drop of each suspension was placed on several parlodion film supports. After all excess fluid had been removed with small capillary pipettes, the films were dried and shadowed with chromium (Williams and Wyckoff, 1945) at arc tangent 2/8 and examined under the RCA electron microscope, type EMU. The balance of the material of each suspension was injected intracerebrally into a *Macaca rhesus* monkey. The monkey injected with the infected mouse brain and cord suspension showed typical poliomyelitis symptoms after a period of 7 days. The monkey was sacrificed. The report of the histopathological studies made in the Armed Forces Institute of Pathology, Washington, D.C., is as follows:

Microscopic: Brain: The large hemorrhage is immediately ventrolateral to the lateral ventricle, being separated from it by a zone of nervous tissue and reactive cells. The hemorrhage is surrounded by a variable cellular zone consisting essentially of macrophages, proliferating glial cells, infiltrating round cells and some neutrophils. A few small foci of neutrophils are seen, usually in the margin of the homorrhage. There is a varible perivascular cuffing with round cells throughout the brain. Foci of glial proliferation are evident.

Spinal Cord: There is an apparent patchy increase of glial elements in the gray matter. Foci of necrosis with glial proliferation are seen, some being rather extensive. Neuronophagia is seen. Satellitosis is evident.

Diagnosis: (1) Hematoma, left basal ganglia of brain, monkey. (2) Polioencephalomyelitis, monkey.

The monkey injected intracerebrally with suspensions of normal mouse brain and cord appeared normal and was discarded after a 30-day observation period.

Upon electron microscope examination of the concentrated suspension of the brain and cord of the normal mice, no virus-like particles could be demonstrated. These controls were

screened with special care. Upon examination of the concentrated suspension from the infected mouse brain and cord, virus-like particles were demonstrated as shown in Fig. 1. Some of the bodies varied from spherical to slightly asymmetrical, ranging in size from 18–20 mu in diameter.

Discussion

In our observation by electron microscopy of the Lansing strain of poliomyelitis virus after passage in the mouse, the results seem to conform to those obtained by Loring *et al.* (1946). In his observation of purified Lansing virus he found that some of the virus particles were asymmetrical and measured 12–34 mu. In previous studies by electron microscopy of the Brunhilde (Reagan *et al.*, 1950) and Leon (Reagan *et al.*, 1951) poliomyelitis strains the Brunhilde was found to resemble the Lansing strain to some extent, but the Leon was rod-like in shape.

Summary

Studies by electron microscopy of brains and spinal cords of mice infected with poliomyelitis virus, Lansing strain, show the virus to be slightly asymmetrical, ranging in diameter from 18–20 mu. These bodies could not be demonstrated in concentrated normal mouse brain and spinal cord subjected to the same procedure of concentration and electron examination.

The bodies from the infected preparation resemble the bodies of purified Lansing poliomyelitis virus demonstrated by Loring.

REFERENCES

Armstrong, C.: Successful Transfer of the Lansing Strain of Poliomyelitis Virus from the Cotton Rat to the White Mouse, ***Pub. Health Repts.***, 1939, *54*, 2302.

Loring, H. S., L. Marton, and C. E. Schwerdt. Electron Microscopy of the Purified Lansing Virus, *Proc. Soc. Exptl. Biol. Med.*, 1946, *62*, 291.

Reagan, R. L., D. M. Schenck, and A. L. Brueckner: Morphological Observations by Electron Microscopy of the Brunhilde Strain of Poliomyelitis Virus, *J. Inf. Dis.*, 1950, *86*, 295.

Reagan, R. L., D. M. Schenck, and A. L. Brueckner: Morphological Observations by Electron Microscopy of the Leon Strain of Poliomyelitis Virus, *Proc. Soc. Exptl. Biol. Med.*, 1951, *77*, 42.

Williams, R. C., and R. W. G. Wyckoff: Electron Shadow Micrography of Virus Particles, *Proc. Soc. Exptl. Med.*, 1945, *58*, 265.

EFFECT OF MERCURIAL DIURETICS UPON THE RESPIRATION OF THE RAT HEART AND KIDNEY

III. The Protective Action of Ascorbic Acid against Mercuhydrin *in vitro*

Arthur Ruskin and Belle Ruskin*

Previous work indicates depression of oxygen uptake of rat heart and kidney slices by rather large amounts of meralluride (Mercuhydrin) and mercaptomerin (Thiomerin) *in vitro* (Ruskin, Nowinski and Ruskin, 1950). This depression of oxidation corresponds to the *in vivo* toxicity of mercurials (Chapman and Shaffer, 1947; Ruskin and Johnson, 1949), as well as their depression of succinic dehydrogenase (Handley and Lavik, 1950), of anaerobic glycolysis (Ruskin, Nowinski and Ruskin, unpublished data, 1950), and of adenosine triphosphatase (Goth, Holman and O'Dell, 1950).

Since ascorbic acid reverses the cardiotoxic effects of mercurials *in vivo* (Chapman and Shaffer, 1947; Ruskin and Johnson, 1949) and, possibly, the enzymotoxic cellular effects of cobalt (Barron and Barron, 1936), pentobarbital (Greig, 1947) and arsenite (Snow and Zilva, 1941), an attempt to show similar protective effects of ascorbic acid against mercurials was in order.

Method of Preliminary Data

The general procedure of measuring the oxygen uptake of rat heart and kidney slices using the Warburg technique was previously described (Ruskin, Nowinski and Ruskin, 1950). Mercuhydrin was added to each flask in the concentration of 5.5×10^{-3}M; in a few instances 2.7×10^{-3}M solutions were employed. Ascorbic acid was used with heart and kidney slices in 2.2×10^{-3}M, 1.1×10^{-2}M, and 4.4×10^{-2}M concentrations, both with and without Mercuhydrin. Concentrations of

*From the Department of Internal Medicine and the Tissue Culture Laboratory of The University of Texas, Medical Branch, Galveston, Texas. Aided by a contract from the Office of Naval Research, N6onr-266 T. O. V., administered by Dr. C. M. Pomerat. Received for publication March 6, 1952.

2.2×10^{-2}M of l-ascorbic acid were also added to heart and kidney (cortex) slices after five-hour incubation to note the additive effect of ascorbic acid on the markedly lowered respiration of these hypoxic tissues.

To obviate the possibility that ascorbic acid detoxifies Mercuhydrin by combining chemically with it, it was attempted to measure the ascorbic acid remaining after its incubation with tissue and Mercuhydrin at various time intervals by the Harris-Birch method. The loss of ascorbic acid so determined was small, but the data were inconclusive. Therefore, ascorbic acid was determined by the more exact ascorbic acid oxidase method of Taubert and Kleiner (1935).

The results of a typical experiment follow: (ml. of dye used).

	Time (minutes)	15	45	75	135	195	495
12 mg.	Ascorbic acid	6.65	6.05	4.45	3.25	2.34	0.10
12 mg.	Ascorbic acid + 1 ml. Mercuhydrin (40 mg. Hg.)	6.50	6.10	5.25	4.35	3.31	0.15

From the above results, which can be interpreted only in relative terms because of variations in oxidation of ascorbic acid, it is clear that no great loss of ascorbic acid occurs. As is probably true for other heavy metals which the Vitamin C detoxifies, any demonstrable chemical combination of ascorbic acid with the mercury compound seems most improbable. Furthermore, any great rise in oxygen consumption upon the addition of ascorbic acid to any system, as noted *infra*, is probably not due to its oxidation *per se*.

Results

Figure 1 indicates that ascorbic acid *per se*, as a reducing agent, uses up oxygen in an oxygen atmosphere in proportion to the amount of ascorbic acid present. Rough indices of its possible bolstering effects, beyond its own oxidation, are seen in figure 1 and Table I. The oxygen uptake of the heart and kidney slices without ascorbic acid, after four-hour incubation, corresponds to one-quarter and two-fifths (cf. Tables II and

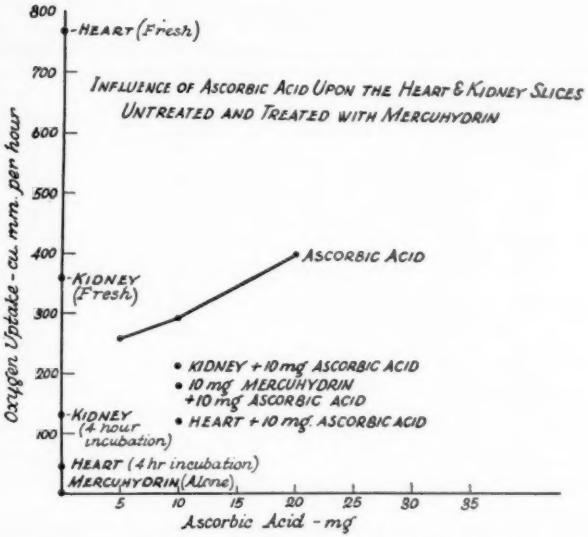

FIG. 1. Oxygen uptake with increasing doses of ascorbic acid; influence of length of incubation upon the oxygen uptake of rat heart and kidney cortex slices; effect upon oxygen uptake of the presence of ascorbic acid on hypoxic (4-hour) kidney, with and without Mercuhydrin added, and heart.

III), respectively, as during the first hour of incubation, however, when ascorbic acid is added, the respiration increases four times in the heart slices and twice in the kidney in the fifth hour of the experiment.

The oxygen consumption of heart slices is raised over 50% by 1.1×10^{-2}M and over 100% by 2.2×10^{-2}M and 4.4×10^{-2}M concentrations of ascorbic acid (Table III). An asymptote in Qo_2 elevation is apparently reached at levels of ascorbic acid of 2.2×10^{-2}M and above. It is even clearer in the case of kidney slices that the slight elevation of oxygen consumption (ca. 18%) by ascorbic acid (Table III) is independent of its concentration and hence, probably, not a purely additive effect.

Table IV indicates the usual (ca. 50%) depression of oxygen consumption of rat heart slices by Mercuhydrin noted previously (Ruskin, Nowinski and Ruskin, 1950). When ascorbic

TABLE I

Respiration of Rat Heart and Kidney slices with and without Ascorbic Acid added after four hours of incubation

Heart		Kidney	
Buffer (Control)	2.2×10^{-2}M Ascorbate	Buffer (Control)	2.2×10^{-2}M Ascorbate
— 0.6	— 6.2	— 7.2	—13.3
— 1.9	— 8.1	— 6.7	—10.3
— 3.7	— 9.3	— 7.4	—12.6
— 3.7	— 8.0	— 6.1	—12.8
— 2.5	—14.4	— 6.3	—14.7
— 1.8	— 9.6	— 7.4	—12.9
— 1.9	—12.0	— 8.1	—13.6
— 1.8	— 6.3	— 5.5	—11.0
— 0.3	— 8.9	— 8.4	—12.9
— 1.2	— 9.2	— 6.2	...
...	— 7.5	...	...
...	— 8.5	...	...
...	— 9.0	...	...
....	— 6.6	...	...
...	— 6.4	...	...
...	— 5.7	...	...
...	—13.5	...	...
Avg. Q_{O_2} — 1.9	— 8.8	— 6.9	—12.7
(No. expts.) (10)	(17)	(10)	(9)

TABLE II

Respiration of Rat Heart Slices with varying amounts of Ascorbic Acid

No Ascorbic Acid	1.1×10^{-2}M Ascorbate	2.2×10^{-2}M Ascorbate	4.4×10^{-2}M Ascorbate
— 7.6	—14.3	—12.2	—14.6
— 9.0	—13.4	—14.4	—15.7
— 7.0	—12.7	—13.1	—12.3
— 7.9	—12.8	—15.3	—17.0
— 7.1	—12.5	—15.5	—17.0
— 8.5	—13.3	—14.0	—12.9
— 6.9	—10.0	—15.7	—10.7
— 6.2	—10.4	—20.2	—18.3
...	—16.7	—15.5	—17.8
...	—10.5	—13.3	—19.2
...	—15.0	—18.2	—15.2
...	—13.6	...	...
...	—11.4	...	...
...	—10.3	...	...
...	— 9.8	...	...
...	—10.0	...	...
...	— 9.0	...	...
...	— 8.2	...	...
...	—10.5	...	...
...	—10.7	...	...
...	— 9.6	...	...
Avg. Q_{O_2} — 7.5	—11.7	—15.2	—15.5
(No. expts.) (8)	(21)	(11)	(11)

TABLE III

Respiration of Rat Kidney slices with varying amounts of Ascorbic Acid

	Control	1.1 x 10^{-2}M Ascorbate	2.2 x 10^{-2}M Ascorbate	4.4 x 10^{-2}M Ascorbate
	—21.8	—23.2	—20.4	—25.6
	—19.1	—22.9	—19.9	—19.5
	—17.5	—21.2	—20.1	—19.7
	—14.2	—19.6	—20.6	—23.3
	—15.5	—18.7	—19.9	—24.5
	—17.0	—24.3	—18.6	—19.3
	—19.1	—16.0	—19.4	—20.9
	—15.0	—18.8	—19.5	—21.6
	—18.4	—16.2	—18.9	—20.1
	—12.0	—19.0	—21.6	—18.6
	—18.5	...	—20.1	—11.4
	...	...	...	—13.7
	...	...	...	—24.0
	...	...	...	—22.4
	...	...	...	—22.2
Avg. Q_{O_2}	—17.1	—20.0	—19.9	—20.5
(No. expts.)	(11)	(10)	(11)	(15)

acid is added with Mercuhydrin, in the proportions of 1 mole to 2.5 (2.2×10^{-3}M to 5.5×10^{-3}M), the Qo_2 is depressed as much as by Mercuhydrin alone. When the molar proportion of ascorbic acid to Mercuhydrin is raised to 4:1 or 5:1, the Qo_2 is raised by over 100% of the controls, more especially above the levels of Qo_2 of heart slices incubated with the same concentrations of ascorbic acid only (cf. Table II).

For kidney cortex slices (Table V), the depression of respiration by Mercuhydrin alone (ca. 50%) compares well with our previous results (Ruskin, Nowinski and Ruskin, 1950). Ascorbic acid added in a molar ratio concentration of 1:2.5 in respect to Mercuhydrin in ineffective in raising the Qo_2. In molar proportions of 4:1 or 5:1, however, the respiration of kidney slices is elevated to the same height as the controls (kidney slices with ascorbic acid only).

Discussion

That mercurial preparations interfere with certain oxidative enzymes in large (Handley and Lavik, 1950; Goth, Holman and O'Dell, 1950), if not therapeutic (Fawaz and Fawaz,

1950), doses, and, consequently, with tissue respiration and anaerobic glycolysis (Ruskin, Nowinski and Ruskin, 1950) has already been noted. It is equally probable that l-ascorbic acid and its analogs increase tissue respiration catalytically. Thus, in liver tissue slices of guinea pigs on restricted diets the Qo_2 is increased from 3 to 4 times as much as would be expected from oxidation (i.e., loss) of the ascorbic acid itself. Since ascorbic acid fails to reverse phlorhizin inhibition of oxygen uptake, yet raises the Qo_2 of cyanide poisoned tissues, Snow and Zilva (1941) postulated a potentiating effect of ascorbic acid on the coupling of phosphorylation with oxida-

TABLE IV

Respiration of Rat Heart slices with varying amounts of Mercuhydrin and Ascorbic Acid

No Merc.		5.5×10^{-3}M Merc.		5.5×10^{-3}M Merc. 2.2×10^{-3}M Asc.	2.7×10^{-3}M Merc. 1.1×10^{-2}M Asc.	5.5×10^{-3}M Merc. 2.2×10^{-2}M Asc.
— 3.2	— 8.3	— 2.9	— 3.5	— 2.8	—15.8	—10.4
— 3.6	—11.2	— 4.2	— 2.7	— 4.3	—18.7	—24.6
— 8.5	— 8.3	— 2.0	— 2.4	— 3.6	—13.9	—18.4
— 4.8	— 7.7	— 2.0	— 3.3	— 4.4	—18.5	—12.8
— 7.2	—10.4	— 2.2	— 4.2	— 3.7	—14.8	—29.8
— 5.6	— 9.6	— 2.0	— 8.8	— 3.0	—12.4	—13.5
— 5.6	— 8.3	—13.9	— 3.6	— 3.1	—17.1	—27.2
— 2.3	— 6.4	— 1.9	— 1.6	— 4.7	...	—20.2
— 3.2	—15.4	— 1.6	— 7.1	...	...	—21.6
— 4.9	—15.6	— 3.6	— 3.1	...	...	—25.7
— 3.5	—13.0	— 1.7	— 3.0	...	...	—15.6
— 2.2	—11.8	— 3.0	— 5.0	...	...	—18.7
— 4.3	— 8.6	— 6.3	—17.6	...	...	—21.5
— 3.2	— 9.5	— 2.2	— 4.5	...	...	—16.5
— 6.7	— 5.3	— 3.4	— 2.5	...	...	—23.9
— 5.8	—17.0	—11.0	...	...	...	—16.4
— 6.2	— 8.0	— 6.7	...	...	...	—13.7
— 7.0	— 7.4	— 3.3	...	...	...	—11.7
— 3.9	— 5.4	— 3.6	...	...	...	—21.3
— 8.3	— 7.6	— 4.3	...	...	...	—15.0
— 4.9	— 9.0	— 2.5	...	...	...	—19.5
—10.3	— 7.0	— 2.1	...	...	...	—21.2
— 4.8	— 7.9	— 4.3	...	...	...	—13.9
—17.0	— 7.1	— 2.6	...	...	...	...
— 5.9	— 8.5	— 5.5	...	...	...	...
— 3.8	— 6.9	— 2.2	...	...	...	...
—13.3	— 6.2	— 2.6	...	...	...	...
— 6.6	...	— 4.0	...	...	...	...
— 4.3	...	— 1.7	...	...	...	...
— 3.0	...	— 3.0	...	...	...	...
Avg. Qo_2 — 7.4		— 4.1		— 3.7	—15.9	—18.8
(No. expts.) (57)		(45)		(8)	(7)	(23)

tion. It was also shown by these workers that increasing concentrations of ascorbic acid increased the Qo_2 up to a maximum level, further increases of the vitamin thereafter being no more effective. These results are consonant with our findings of similar asymptotic levels of ascorbate concentrations of 2.2×10^{-2}M for heart and 2.2×10^{-3}M for kidney slices (Tables II, III and unpublished data).

As indicated above, ascorbate probably catalyes the enzymes of aerobic carbohydrate metabolism. If one were to assume

TABLE V

Respiration of Rat Kidney slices with varying amounts of Mercuhydrin and Ascorbic Acid

No Merc.		5.5×10^{-3}M Merc.	5.5×10^{-3}M Merc. 2.2×10^{-3}M Asc.	5.5×10^{-3}M Merc. 1.1×10^{-2}M Asc.	2.7×10^{-3}M Merc. 1.1×10^{-2}M Asc.	2.7×10^{-3}M Merc. 2.2×10^{-3}M Asc.	5.5×10^{-3}M Merc. 4.4×10^{-2}M Asc.
−12.1	−21.8	− 7.4	− 7.0	−15.6	−14.8	−20.0	−21.1
−15.9	−19.1	− 5.6	−13.1	−10.3	−15.7	−19.8	−20.3
−11.2	−17.5	−13.1	− 8.2	−14.9	−11.1	−17.4	−20.7
−15.7	−14.2	− 6.9	− 8.8	−12.3	−13.7	−17.7	−22.1
−14.3	−15.5	− 6.2	− 8.1	−12.7	−15.8	−21.4	−19.7
−10.6	−17.0	− 7.5	− 9.1	−15.2	−16.3	−19.8	−22.8
−16.7	−19.1	− 9.4	...	−14.9	−13.1	−18.1	−20.1
−16.6	−15.0	− 7.1	...	...	−11.8	−22.7	−18.3
−21.5	−18.4	− 7.2	...	...	−18.0	−21.2	−20.5
−25.2	−12.0	− 6.5	...	...	−18.8	−21.7	−27.2
−14.3	−18.5	− 5.4	...	...	−14.9	−17.6	−23.8
−11.9	−16.2	− 6.0	...	...	−18.5	−19.8	−24.4
−18.1	...	− 6.0	...	...	−16.1	−18.2	−20.9
−11.3	...	− 5.9	...	...	−16.0	−20.2	−21.7
−17.0	...	− 5.6	...	...	−15.5	−18.1	−16.0
−17.1	...	− 8.1	...	...	−13.7	−13.2	−28.6
...	...	...	...	...	−12.1	...	...
...	...	...	...	...	−15.8	...	...
Av. Qo_2	−16.2	− 7.1	− 9.1	−13.7	−15.1	−19.2	−21.8
(No. expts.)	(28)	(16)	(6)	(7)	(18)	(16)	(16)

that the ascorbic acid raised the Qo_2 in purely additive fashion, an assumption which we disproved, one would still expect lower Qo_2 figures for Mercuhydrin plus ascorbic acid than for controls (ascorbic acid alone). Instead, the Qo_2 figures for Mercuhydrin-ascorbic acid-kidney slices are the same as, and for heart slices possibly even higher than, those obtained when ascorbic acid alone is added.

The relatively slight increase in Qo_2 of kidney slices upon the addition of ascorbic acid in our experiments is paralleled by the slight increase in oxygen consumption of liver slices with ascorbate added in the experiments of Snow and Zilva (1941). On the other hand, heart slices (Table II), particularly heart slices after prolonged incubation (Table I), just as vitamin C-deficient guinea pig liver slices (Snow and Zilva, 1941), show much more marked increases in oxygen consumption with the addition of ascorbic acid. As noted above, the Mercuhydrin-poisoned kidneys' Qo_2 is also raised only slightly by ascorbic acid (Table V), whereas the Qo_2 of Mercuhydrin-treated hearts is elevated markedly when sufficient ascorbic acid is present.

The above findings are of interest from two standpoints: first, the heart, on a 24-hour contraction schedule, extracts more oxygen (has a much lower venous oxygen saturation) (Bing, *et al.*, 1949) than liver, kidney, or skeletal muscle. Hence, heart slices fall off much more rapidly than kidney slices in oxygen consumption with time (fig. 1), i.e., suffer more from hypoxia. Secondly, it is much less clear why ascorbic acid should bolster the aerobic metabolism of the heart more than that of the kidney (or liver).

In tissue culture, too, ascorbic acid stimulates growth of chick embryo heart fragments (work in progress), although its effect on mercurialized heart fragments is uncertain. Further studies on the effect of Mercuhydrin and its antagonists on single enzymes involved in these processes are in progress.

Summary and Conclusions

1. Mercuhydrin lowering of oxygen uptake of rat heart and kidney slices, as measured by the Warburg technique, is prevented by ascorbic acid.

2. The elevation of Qo_2 by ascorbic acid, with or without Mercuhydrin, is much greater for heart than for kidney slices.

3. Ascorbic acid, a reducing agent, uses up some oxygen per se. However, its slight loss after incubation can account for only a small portion of the oxygen uptake. Furthermore, its continued presence following incubation with Mercuhydrin speaks against inactivation of the latter by chemical combination of the two compounds.

4. The exact mechanism of potentiation of tissue respiration by ascorbic acid in the presence of deleterious doses of Mercuhydrin is unknown.

5. The ascorbate reversal of the deleterious effects of Mercuhydrin upon the tissue oxygen uptake is in line with the known protective effects of ascorbic acid against heavy metal toxicity *in vivo*.

REFERENCES

Barron, A. G., and Barron, E. S. G.: Mechanism of cobalt polycythemia. Effect of ascorbic acid. *Proc. Soc. Exp. Biol. and Med.*, 35:407–409, 1936.

Bing, R. J., Hammond, W. M., Handelsman, J. C., Powers, S. R., Spencer, F. C., Eckenhoff J. E., Goodale, W. T., Hafkenschiel, J. H. and Kety, S. S.: The measurement of coronary blood flow, oxygen consumption and efficiency of the left ventricle in man. *Am. Heart J.*, 38:1–24, 1949.

Chapman, D. W. and Shaffer, C. F.: Mercurial diuretics; comparison of acute cardiac toxicity in animals and effect of ascorbic acid on detoxification in their intravenous administration. *Arch. Int. Med.*, 79:449–456, 1947.

Fawaz, G. and Fawaz, E. N.: Mechanism of action of mercurial diuretics. *Proc. Soc. Exp. Biol. and Med.*, 77:239–241, 1951.

Goth, A., Holman, G. and O'Dell, O. V.: Effect of mercurials on kidney adenosine triphosphatase activity. *Proc. Soc. Exp. Biol. and Med.*, 74: 178–180, 1950.

Greig, M. E.: The effect of ascorbic acid in reducing the inhibition of brain metabolism produced by pentobarbital in vitro. *J. Pharmacol. and Exp. Therap.*, 91:317–323, 1947.

Handley, C. A. and Lavik, P. S.: Inhibition of the kidney succinic dehydrogenase system by mercurial diuretics. *J. Pharmacol. and Exp. Therap.*, 100:115–118, 1950.

Ruskin, A. and Johnson, J. E.: Cardiodepressive effects of mercurial diuretics; cardioprotective value of BAL, ascorbic acid, and thiamin. *Proc. Soc. Exp. Biol. and Med.*, 72:577–583, 1949.

Ruskin, B., Hsu, T. C., Pomerat, C. M. and Ruskin, A.: Unpublished data, 1951.

Ruskin, B., Nowinski, W. W. and Ruskin, A.: Effect of mercurial diuretics upon the respiration of the rat heart and kidney. I. Effect of Mercuhydrin and its fractions. *Tex. Rep. Biol. and Med.*, 8:384–390, 1950.

Ruskin, B., Nowinski, W. W. and Ruskin, A.: Effect of mercurial diuretics upon the respiration of rat heart and kidney slices. II. The effect of thiomerin. *Tex. Rep. Biol. and Med.*, 8:391–394, 1950.

Ruskin, B., Nowinski, W. W. and Ruskin, A.: Unpublished data, 1950.

Snow, G. A., and Zilva, S. S.: The action of l-ascorbic acid on the in vitro respiration of liver tissue from guinea-pigs on a restricted diet. II. *Biochem. J.*, 35:787–800, 1941.

Taubert, H. and Kleiner, I. S.: An enzyme method for the estimation of true vitamin C. *J. Biol. Chem.*, 110:559–563, 1935.

COMPARATIVE EFFECTS OF VERTAVIS,* SODIUM DEPLETION, AND SODIUM REPLETION IN ESSENTIAL HYPERTENSION

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Our preliminary reports (Ruskin and Rider, 1950) have affirmed the hypotensive effects of both Vertavis and sodium depletion in essential hypertension. The hypotensive action of veratrum viride and its alkaloids has been both claimed (Wilkins, 1951; Freis, etc., 1949; Meilman and Krayer, 1950) and, except in toxic doses (Coe, *et al.*, 1950), denied. Many investigators have, however, used crude "before and after" comparisons of blood pressure readings, criticized by us (Ruskin and McKinley, 1947).

The recent reintroduction of salt depletion regimes, involving simple sodium restriction (Grollman, *et al.*, 1945; Bryant and Bleche, 1947) the rice-fruit diet (Kempner, 1944) or marked sodium restriction (200 mg. daily) plus mercurial natruresis (Megibow, *et al.*, 1948) have often resulted in some depression of the blood pressure level in hypertensive patients. On the other hand, excessive salt intake alone had mostly failed to alter hypertensive blood pressures, although the simultaneous administration of desoxicorticosterone and salt often raises them in hypertensive (Perera and Blood, 1946, 1947) and hypotensive (Ruskin, *et al.*, 1948) cases.

In this study we have attempted to reduce high arterial pressures by stringent prolonged sodium depletion, by Vertavis administration, and by combined regimens; to compare the therapeutic and toxic effects of the three modes of treatment; and to evaluate the possible pressor effects of marked sodium repletion—in the same group of cases of essential hypertension.

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*Bioassayed Vertatrum viride, Irwin Neisler & Co., Decatur, Ill. From the Departments of Internal Medicine and Public Health, The University of Texas Medical Branch, and the John Sealy Hospital, Galveston, Texas. Submitted for publication January 15, 1952. This study was aided by a grant from Irwin Neisler & Co. We are grateful to Dr. E. J. Poth for the use of the facilities of the Experimental Surgery Laboratory.

Material and Method

Thirty-one cases of essential hypertension, excluding those with cardiac or renal insufficiency, were followed twice weekly in the Hypertensive Clinic.

All regimens lasted three weeks each. In one such period Vertavis was administered, starting with 10 Craw units twice daily, increasing by 10 units hourly to tolerance, the maximum dosage varying between 20 and 100 (usually 40 to 60) units daily. Patients on Vertavis were examined 3 to 4 hours after the first morning dose, at the time of the expected maximum hypotensive effect. In a second period, sodium depletion was accomplished by a 200 mg. sodium diet and the subcutaneous administration of 2 ml. Thiomerin or Mercuhydrin twice weekly. A third regimen involved the use of both Vertavis and sodium depletion as described above. In a fourth period sodium repletion was produced by equal divided doses of sodium chloride and sodium bicarbonate totalling 24 grams daily. A fifth period of three weeks served as a control period during which lactose tablets were administered in a dosage of 3 to 9 a day.

At every visit the brachial blood pressures were recorded in triplicate after rest in the sitting position. For each regimen 36 blood pressure readings were thus available for statistical evaluation. Symptoms and signs were noted. Serum and 24-hour urine sodium levels were estimated by flame photometry and sodium clearances* were calculated.

The method of comparing the medians and means of all the blood pressures taken in any therapeutic period with those of the placebo period was essentially the same as that used in our previous study of possible hypotensive drugs (Ruskin and McKinley, 1947). This procedure was again found to be vastly superior to the "before and after" method, with relatively few control readings, used by most workers. The results were quite comparable to those obtained by utilizing basal blood pressure readings (Perera and Blood, 1946, 1947).

In 13 patients vital capacities were taken in triplicate each week, 9 readings per regimen. In 10 others, Etamon tests

*U V/P (V (ml./min.) obtained from the 24-hour urine volumes).

(0.7 mg./kg. intravenously) were performed at least once during each regimen—Vertavis, sodium depletion, and sodium repletion.

Results

Symptomatic Effects. From Table I it will be noted that dizziness, and the appearance and disappearance of headache, occurred under all regimens, including the placebo period. Suggestively, headaches disappeared relatively more often during the low sodium regime, and appeared more commonly on marked sodium repletion. Dizziness was present with equal frequency in both the high and the low sodium periods. Weakness and fatigue-ability, and muscle cramps were prominent during the Vertavis and sodium depletion regimens. These symptoms are characteristic of the early stages of the "low salt syndrome" (Schroeder, 1949). Weight loss of as much as 2 to 4 kg. occurred particularly on sodium depletion.

TABLE I

Clinical findings on various regimens in 31 cases of essential hypertension

	Placebo	Vertavis	Low Sodium	Vertavis & Low Sodium	High Sodium
Headache appeared	2	5	2	4	12
Headache disappeared	6	8	11	4	2
Dizziness	4	8	10	8	12
Syncope	0	2	1	2	1
Weakness and fatigue	1	7	10	11	3
Salivation	0	14	0	4	0
Abdominal and leg cramps	0	3	9	6	1
Nausea	2	14	6	10	6
Nausea and vomiting	2	18	6	10	7
Diarrhea	0	1	5	3	1
Dyspnea and orthopnea	3	3	0	1	9
Precordial or epigastric pain	3	2	1	2	14
Polydipsia and polyuria	0	0	1	0	17
Edema	1	3	0	0	11
Congestive failure	0	0	0	0	3

Salivation was characteristic of, and nausea and vomiting most prominent in, the Vertavis and combined Vertavis-sodium depletion periods. Although dyspnea, orthopnea, precordial or epigastric pain, and, in 4 cases, edema occurred without frank

congestive failure on placebo therapy and on Vertavis administration, they were conspicuously absent during sodium depletion, and much more frequently present during the sodium repletion regimen.

Sodium repletion resulted, on the one hand, in polydipsia, and, on the other hand, in 3 cases, in oliguria and frank congestive failure (cases R. B., A. C., and J. W., Tables II, VI, and VII). Gain in weight was the rule during the high sodium period, and amounted to as much as 3 to 5 kilograms.

Vital capacities, as rough indices of pulmonary vascular congestion, did not seem to be affected in any regular fashion. Table II indicates that they were occasionally higher on sodium depletion and lower on sodium repletion, to be expected from the relative hypovolemia and hypervolemia of the two regimens. Drastic falls in vital capacity in the high sodium periods, as in patients L. C. and D. W. (Table II), may have signified latent congestive heart failure.

TABLE II

Vital capacities in 13 patients with essential hypertension on marked sodium depletion and repletion and Vertavis regimens
Vital Capacity "ceilings"

Patients	Low Sodium	High Sodium	Vertavis
1. A.B.	98%	98%	98%
2. R.B.	81%	66%	72%
3. M.C.	80%	68%	62%
4. L.C.	78%	69%	83%
5. L.D.	71%	71%	71%
6. S.G.	80%	74%	74%
7. G.H.	79%	73%	67%
8. J.H.	70%	70%	76%
9. M.J.	72%	70%	72%
10. V.R.	89%	102%	95%
11. W.S.	54%	64%	49%
12. D.W.	83%	63%	70%
13. J.W.	107%	70%	107%
Lowest V.C. levels— No. cases	1	5	3

Blood Pressure Effects. The changes on various regimens of the systolic (Table III) and diastolic (Table IV) arterial pressures from the placebo period levels were, in general, parallel, with greater scatter in the systolic pressures.

TABLE III

Systolic blood pressure changes from placebo levels in 31 cases of essential hypertension following Vertavis, marked sodium depletion; combined Vertavis and sodium depletion and sodium repletion

Regimen	−30–40mm.	−21–30mm.	−11–20mm.	−1–10mm.	+0–10mm.	+11–20mm.
Vertavis	3	12	8	5	3	0
Sodium depletion	3	16	9	2	1	0
Vertavis and sodium depletion	6	15	9	1	0	0
Sodium repletion	0	0	4	6	18	3

TABLE IV

Diastolic blood pressure changes from placebo levels in 31 cases of essential hypertension following Vertavis, marked sodium depletion, combined Vertavis and sodium depletion, and sodium repletion

Regimen	−21–30 mm.	−11–20 mm.	−1–10 mm.	+0–10 mm.
Vertavis	0	8	18	5
Sodium depletion	4	14	12	1
Vertavis and sodium depletion	6	19	6	0
Sodium repletion	0	0	12	19

On Vertavis, the median falls were from −21 to −30 mm. in the systolic pressures, and from −1 to −10 mm. in the diastolic pressures. Statistically significant systolic and diastolic hypotensive effects of Vertavis at the 1% level ($P < 0.01$) were noted in 20 of 31 patients, or 64.5%.

On the sodium depletion regimen, the median falls were from −21 to −30 mm. in the systolic pressures, and from −11 to −20 mm. in the diastolic pressures. In 4 cases the diastolic pressures decreased as much as −21 to −30 mm. Statistically significant diastolic hypotensive effects ($P < 0.01$) were found in 24 of 31 cases, or 77.4%.

The combined Vertavis-sodium depletion regimen produced the same median falls in diastolic and systolic pressures as the sodium depletion period alone. However, there were 2 more cases showing diastolic hypotensive effects in the −1 to 10 mm. range, and 5 more in the −11 to 20 mm. range.

Sodium repletion periods, when contrasted with placebo management, revealed minimal rises (+ 0 to 10 mm.) in the systolic and diastolic pressures in the majority of cases. Slight

falls occurred in a smaller group of patients. Statistically significant rises in the diastolic pressure occurred in only 8 of 31 patients, or 25.8%; significant falls in 3 of the 31, or 9.7% of the cases.

Etamon "Floors." In 10 cases 0.7 mg./kg. of tetraethyl ammonium chloride was injected intravenously, at least once during each one of the Vertavis, low sodium and high sodium periods (Table V). The most marked falls in the blood pressure occurred during the sodium depletion regimen in 6 of the 10 cases, most striking in patients S. G., J. H., and A. B. However, with marked sodium repletion, intravenous Etamon still produced marked hypotension (supine and orthostatic) in the majority of the cases. Two cases actually showed the most striking diastolic hypotension as a result of this variable test during the period of sodium redundance. In two other cases, the Vertavis period was associated with the most marked hypotensive reaction to Etamon. There was no definite correlation of Etamon hypotension with the serum sodium levels or the patients' weights.

TABLE V

Etamon tests in 10 patients with essential hypertension on marked sodium depletion and repletion and Vertavis regimens

Patient	Etamon "floor"		
	Low Sodium	High Sodium	Vertavis
1. S.G.	50/0	125/70	140/100
2. J.H.	80/10	135/80	140/70
3. A.B.	70/60	100/60	110/80
4. W.S.	130/75	150/80	135/80
5. M.J.	150/80	140/85	200/100
6. G.H.	120/80	170/100	140/70
7. D.W.	150/90	150/90	120/80
8. L.D.	130/85	130/70	170/90
9. V.R.	130/100	145/95	190/130
10. R.B.	180/135	175/100	210/140
Lowest Etamon floors—No. cases	6	2	2

Effects on Serum and Urine Sodiums. From Table VI and Fig. 1 it will be seen that no drastic effects on serum sodium were produced by any of the regimens. There was a tendency for serum sodium to fall, even below "normal," especially on

combined Vertavis administration and sodium depletion. Lesser effects in this direction were occasionally noted in the sodium depletion and the Vertavis periods. Similarly, rises of serum sodium on sodium repletion were sporadic and borderline.

TABLE VI

Serum sodium levels (normal: 135 to 150 milliequivalents per liter) in 31 cases of essential hypertension on various regimens

Sodium Level	Vertavis	Low Sodium	Vertavis and Low Sodium	High Sodium
Low	18	42	87	5
Normal	76	53	12	80
High	5	4	0	14
Total	99	99	99	99

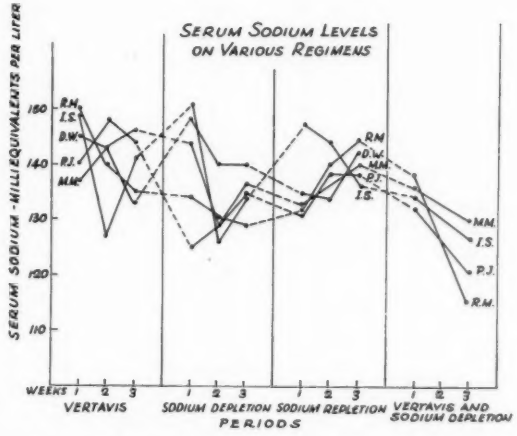

FIG. I. Serum sodium levels on various regimens in 5 representative cases.

Sodium clearances (Table VII were definitely subcontrol with oliguria, during sodium depletion, and often double the control levels, with polyuria, in the course of sodium repletion. In more than occasional cases (e.g., cases A. C., L. C., M. G., M. M., and I. S., Table VII), Vertavis administration seemed

to be associated with increased sodium excretion. Of these, only case A. C. developed frank congestive heart failure on sodium repletion, and in the two others that did so (A. B. and J. W.) Vertavis had no apparent natruretic effect.

TABLE VII

Serial weekly serum sodium levels (milliequivalents per liter), parallel sodium clearances (24 hour urines), and diastolic blood pressure changes from the controls on various regimens in representative cases.

	Vertavis			Sodium depletion			Sodium repletion		
Case	Serum Sodiums	Sodium Clear's	Changes D.P.mm.	Serum Sodiums	Serum Clear's	Changes D.P.mm.	Serum Sodiums	Sodium Clear's	Changes D.P.mm.
	146	0.87		136	0.58		142	1.48	
3. R.B.	147	0.93	+ 5	127	0.74	—30	145	1.89	+ 5
	137	1.07		118	0.46		155	1.56	
	149	0.79		128	0.59		141	2.42	
4. A.B.	132	1.28	—10	144	0.45	—20	134	2.46	—10
	136	1.58		138	0.48		136	2.24	
	148	1.28		144	0.48		138	1.98	
5. R.B.T.	150	1.35	—20	141	0.35	—10	142	1.60	— 5
	144	1.45		132	0.39		142	1.66	
	154	0.83		150	0.36		141	2.07	
6. O.C.	154	1.33	—20	141	1.21	—20	143	2.70	+ 5
	147	0.98		144	0.71		152	2.27	
	151	0.94		129	0.30		145	2.04	
9. A.C.	142	2.26	—10	145	0.57	—15	138	1.85	— 5
	143	1.33		140	0.57		154	1.78	
	135	1.26		154	0.48		142	1.81	
10. L.C.	139	1.58	—10	147	0.61	—30	138	1.71	—10
	133	1.79		138	0.78		143	1.62	
	155	0.87		140	0.32		135	2.08	
11. E.D.	150	1.27	—15	132	0.31	—30	142	1.33	—10
	142	1.28		128	0.54		148	1.68	
	142	1.67		128	0.67		128	3.13	
15. M.G.	146	2.27	—10	127	0.92	—10	147	1.79	— 5
	145	1.56		137	0.39		145	2.12	
	147	0.92		144	0.26		140	2.31	
17. J.H.	149	0.85	—10	141	0.26	—10	138	1.52	+10
	143	1.05		130	0.47		143	1.25	
	150	1.35		143	0.38		133	2.36	
18. G.H.	139	1.57	—10	147	0.73	—30	139	2.01	+ 5
	141	1.21		127	0.93		141	1.95	
	140	0.80		125	0.28		131	1.86	
21. P.J.	148	1.04	—10	129	0.33	—10	138	1.69	— 5
	144	1.12		135	0.27		138	1.10	

TABLE VII—Continued

Serial weekly serum sodium levels (milliequivalents per liter), parallel sodium clearances (24 hour urines), and diastolic blood pressure changes from the controls on various regimens in representative cases.

Case	Serum Sodiums	Sodium Clear's	Changes D.P.mm.	Serum Sodiums	Serum Clear's	Changes D.P.mm.	Serum Sodiums	Sodium Clear's	Changes D.P.mm.
		Vertavis			Sodium depletion			Sodium repletion	
	140	1.30		135	0.23		144	1.31	
L.J.	148	0.71	—10	134	0.14	—10	154	1.64	+ 5
	144	0.73		146	0.83		146	1.55	
	144	1.29		142	0.82		139	1.97	
J.L.	122	1.51	—10	137	1.00	—15	142	2.24	— 5
	138	1.55		128	1.33		147	2.14	
	137	1.04		144	0.19		133	1.71	
M.M.	143	2.57	—10	126	0.38	—10	136	2.23	— 5
	146	1.53		134	0.41		140	1.69	
	149	1.38		134	0.19		134	1.94	
R.M.	140	1.43	—10	130	0.31	—10	140	1.81	+ 5
	135	1.04		129	0.46		144	1.90	
	149	1.84		151	0.64		142	1.38	
I.S.	127	1.97	—10	126	0.87	—20	144	1.76	— 5
	141	2.18		134	0.75		136	2.43	
	148	1.49		130	0.31		136	2.25	
L.V.	151	1.07	—15	136	0.45	—25	138	2.22	— 5
	132	1.51		131	0.38		147	1.67	
	142	1.08		134	0.31		144	1.62	
J.W.	151	1.17	—15	143	0.60	—25	143	1.50	+10
	125	0.85		119	0.85		151	1.52	
	145	0.79		147	0.25		135	1.10	
D.W.	143	0.94	—10	140	0.45	—15	134	1.68	— 5
	133	1.08		140	0.50		142	1.88	

Discussion

Blood Pressure and Serum Sodium Levels. The serum sodium levels of our cases of essential hypertension were essentially normal, as noted by others (Holley, Elliott, Holland, 1951). Hypotensive effects of Vertavis administration were statistically proven in two-thirds of the cases of essential hypertension. Marked sodium depletion—restriction of sodium intake plus mercurial natruresis—was only slightly more effective. On occasion, the most marked blood pressure drops were

obtained by the combined Vertavis and sodium depletion regimen. There were suggestive falls in serum sodium levels in line with these hypotensive effects (Fig. 1), but no close correlation between these two phenomena was noted.

Of importance was the finding of actual rises in arterial pressure, if only statistically valid in one quarter of the cases, on sodium repletion. This is proof that elevation of hypertensive blood pressures may occur on a very high salt intake alone in humans, as has been claimed for rats (Sapirstein, 1950) and occasionally for dogs (Wilhelmj, 1951). We were able to attain such high sodium intakes without major gastric upsets by concommitant administration of sodium bicarbonate with sodium chloride.

That the blood pressure changes on salt repletion and depletion were no greater than those found, could be explained on the basis of adrenocortical and other homeostatic mechanisms. While serum sodium levels rose occasionally, there was no definite correlation between them and the rises in blood pressure on the salt repletion regimen. Changes in the interstitial and intracellular sodium and other cations may, however, be of much greater importance in this respect. Salt loading probably results in some sodium retention in hypertensive individuals, if less than in those with congestive heart failure (Gregory, *et al.*, 1950). The suggestion that the supernormal sodium content of arterial walls in hypertensive subjects may promote swelling and narrowing of the arterioles (Tobian and Binion, 1951) is also of interest in connection with the possible hypertensive effects of salt repletion and the hypotensive effects of salt depletion.

Other Effects. Symptomatically, in some cases, Vertavis was responsible particularly for unpleasant salivation, nausea, vomiting, dizziness, and weakness; sodium depletion—for weakness, dizziness, abdominal and leg cramps, nausea, vomiting, and diarrhea. The occasional natruretic effect of prolonged administration of Vertavis is unexplained, except, possibly, on the basis of the cardiotonic effects of veratrum alkaloids in cases of latent congestive failure. Acute experiments, employing intravenous Verenteral (Lot 33 X), indicate parallel hypotensive, gastrointestinal, and diminished renal function and

urinary volume and cation output effects (Ruskin and Ruskin, unpublished). Perhaps also, prolonged administration of Vertavis evokes at times hormonal and intrarenal mechanisms productive of excessive excretion of salt. This remains to be proven.

Sodium depletion was also most effective in relieving headache, and avoiding dyspnea and orthopnea (latent pulmonary congestion?) and peripheral edema. Sodium clearances fell, presumably as a result of prolonged sodium starvation and in line with the findings of others that sodium excretion is not raised by sodium restriction in hypertensive patients as it is in normal individuals.

Neither marked sodium depletion, nor the combined Vertavis-sodium depletion regimen resulted, however, in the full-blown picture of the "low salt syndrome." While hyponatremia was common and the urinary output was often decreased, there were no significant rises in the blood non-protein nitrogen or its components or other evidences of progressive renal insufficiency. The original adequacy of renal function, as compared with its initial impairment in cases of congestive heart failure suffering from the "low salt syndrome," may be responsible for the resistance of early hypertensive patients to the ill effects of sodium depletion.

Sodium repletion was not infrequently responsible for polydipsia, polyuria, precordial or epigastric pain, dizziness, nausea, vomiting, peripheral edema, and dyspnea and orthopnea. The excessive salt intake, despite compensatory antidiuretic hormone oversecretion, was conceivably responsible for thirst, polyuria, and natruresis. Frank congestive failure developed in at least three instances, and if circulation time studies had been done, perhaps more cases of myocardial insufficiency would have been identified. The comparative vital capacity determinations were of relatively little value in this respect. If, despite increased sodium excretion (Table VII), sodium retention occurred, as it may do on salt loading in hypertensive individuals free from myocardial or frank renal insufficiency, then it is not surprising that various phenomena resulting presumably from increased interstitial fluid and plasma volumes ensued, to the extent of hypervolemic heart failure. The

congestive phenomena were unrelated to the level of aterial pressure (cf. case of R. B., Table V).

Summary

1. Vertavis, oral preparation of Veratrum viride, was effective in significantly ($P < 0.01$) lowering the diastolic blood pressure in 20 of 31 cases of essential hypertension. Frequent untoward effects were gastro-intestinal disturbances, dizziness, and weakness.

2. Marked sodium depletion by means of a 200 mg. (9 milli-equivalent) sodium diet and bi-weekly mercurial diuresis resulted in significant lowering of the diastolic arterial pressure in 24 of the 31 cases. Loss of weight was marked. The "low salt syndrome" ensued in mild forms only.

3. Slightly greater lowering of the blood pressure occurred in 7 instances on the combined Vertavis-sodium depletion regimen. Side effects were frequent.

4. Sodium repletion, consisting of 12 grams each of sodium chloride and sodium bicarbonate daily (ca. 300 milli-equivalents of sodium), produced significant rises in the diastolic blood pressure over placebo levels in 8 of 31 cases, balanced by falls in 3 others. Gains of 3 to 5 kg. in weight, edema, symptoms suggestive of pulmonary and cerebral engorgement, and, in 3 cases, frank congestive heart failure resulted on this regimen.

REFERENCES

Bryant, J. M., and Belche, E.: Low Sodium-Forced Fluid Management of Hypertentive Vascular Disease and Hypertensive Heart Disease, *Proc. Soc. Exp. Biol. and Med.*, 65:227, 1947.

Coe, W. S., Best, M. M., and Kinsman, J. M.: Varatrum Viride in the Treatment of Hypertensive Vascular Disease, *J.A.M.A.*, 143:5, 1950.

Freis, E. D., Stanton, J. R., Culbertson, J. W., Leiter, J., Halperin, M. H., Barnett, C. H., and Wilkins, R. W.: The Hemodynamic Effects of Hypotensive Drugs in Man. I. Veratrum Viride, *J. Clin. Invest.* 28:353, 1949.

Gregory, R., Levine, H., Adams, D. B., and Stembridge, V.: Renal Capacity of Normal, Hypertensive, and Cardiac Failure Patients to Excrete Sodium, *Proc. Centr. Soc. Clin. Res.*, 22:31, 1950.

Grollman, A., Harrison, T. R., Mason, M. F., Baxter, J., Crampton, J., and Reichsman, F.: Sodium Restriction in the Diet for Hypertension, *J.A.M.A.*, 129:533, 1945.

Holley, H. L., Elliott, H. C., Jr., and Holland, C. M., Jr.: Serum Sodium Values in Essential Hypertension, *Proc. Soc. Exp. Biol. and Med.*, 77: 561, 1951.

Kempner, W.: Treatment of Kidney Disease and Hypertensive Vascular Disease with Rice Diet, *No. Carol. Med. J.*, 5:125, 1944.

Megibow, R. S., Pollack, H., Stollerman, J. A., Boston, E. H., and Bookman, J. J.: The Treatment of Hypertension by Accelerated Sodium Depletion, *J. Mt. Sinai Hosp.*, 15:233, 1948.

Meilman, E., and Krayer, O.: Clinical Studies on Veratrum Alkaloids. I. The Action of Protoveratrine and Veratridine in Hypertension, *Circ.*, 1:204, 1950.

Perera, G. A., and Blood, D. W.: Disturbances in Salt and Water Metabolism in Hypertension, *Am. J. Med.*, 1:602, 1946.

Perera, G. A., and Blood, D. W.: Pressor Activity of Desoxycorticosterone Acetate in Normotensive and Hypertensive Subjects, *Ann. Int. Med.*, 27:401, 1947.

Ruskin, A., and McKinley, W. F.: Comparative Study of Potassium Thiocyanate and Other Drugs in the Treatment of Essential Hypertension, *Am. Heart J.*, 34:691, 1947.

Ruskin, A., Roosth, H., and Griffin, H. B.: Effects of Vasoconstrictive and Hypervolemic Measures upon Tetraethyl Ammonium Orthostatic Hypotension, *Amer. J. Med.* (Abstr.), 5:315, 1948.

Ruskin, A., and Rider, A. J.: Veratrum Viride (Bioassayed) in Treatment of Essential Hypertension, *Tex. State J. Med.*, 46:80, 1950.

Ruskin, A., Rider, A. J., Ruskin, B., Scott, J. A., and Rabinowitz, H.: Comparative Effects of Vertavis, Sodium Depletion, and Sodium Repletion in Essential Hypertension, *Am. J. Med.* (Abstr.) 9:264, 1950.

Ruskin, A., and Ruskin, B.: Unpublished Data.

Sapirstein, L. A., Brandt, W. L., and Drury, D. R.: Production of Hypertension in the Rat by Substituting Hypertonic Sodium Chloride Solutions for Drinking Water, *Proc. Soc. Exp. Biol. and Med.*, 73:82, 1950.

Schroeder, H.: Renal Failure Associated with Low Extracellular Sodium Chloride. The Low Salt Syndrome, *J.A.M.A.*, 141:117, 1949.

Tobian, L., Jr., and Binion, J.: Tissue Sodium, Potassium, and Magnesium in Human Subjects, *Proc. Nat. Meet. Amer. Fed. Clin. Res.*, May, 1951, p. 1.

Wilhelmj, C. M., Waldman, E. B., and McGuire, T. F.: Effects of Prolonged High Sodium Chloride Ingestion and Withdrawal upon Blood Pressure of Dogs, *Proc. Soc. Exp. Biol. and Med.*, 77:379, 1951.

Wilkins, R. W.: Veratrum Viride in Hypertensive Cardiovascular Disease, *Mod. Conc. Cardiocasc. Dis.*, 20:89, 1951.

TISSUE CULTURE METHODS IN RELATION TO BIOCHEMICAL STUDIES*

THEODORE WINNICK

The researches of numerous investigators through the years have imparted an extensive and varied knowledge of conditions associated with the growth of isolated animal tissues. Obviously, microscopic examination has afforded the simplest and most reliable qualitative tool for observing growth. Attempts to employ area measurements and wet weights of tissues have not led to adequately quantitative procedures to date.

While relatively few biochemists have ventured into the field, tissue culture methods appear to be excellently adapted to metabolic studies, and are beginning to receive attention in this regard.

Nucleic Acids

Several investigations have been made on the nucleic acid fractions of tissue cultures. An impetus has been the suggestion of Boivin, Vendrely, and Vendrely (1948) that the quantity of desoxypentose nucleic acid (DNA) per nucleus is constant for the somatic cells of a given species. Accordingly, changes in the quantity of DNA would be a sensitive index of cell production or destruction. Pentose nucleic acid (PNA) is found primarily in the cytoplasm of animal cells, and its measurement is equally valuable.

Davidson and Waymouth (1949) determined initial nucleic acid values by analysis of their heart cultures, washed for two days to remove cells damaged during explantation. Subsequently, marked rises in nucleic acid levels resulted. Hull and Kirk (1950A) took the time of explantation as their starting point, and were able to demonstrate a 50 per cent increase in both DNA and PNA content of embryonic chick heart cultures after six days.

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Attempts to employ inorganic radioactive phosphate (labeled with P^{32}) as a tool for measuring nucleic acid synthesis have not been wholly successful, inasmuch as turnover or replacement processes are complicating factors. Hull and Kirk (1950B) found very high rates of P^{32} incorporation into the nucleic acids of embryonic chick heart cultures, when embryo extract-horse serum medium was employed. However, a certain lesser degree of uptake of the labeled phosphate occurred when the latter was employed in a Tyrode medium, in which no growth resulted.

In similar experiments, Gerarde, Jones, and Winnick (1952A) observed a degree of P^{32} uptake into DNA corresponding to a 60 per cent turnover, with embryonic lung cultures cultivated in embryo extract medium for two weeks. The turnover of phosphate in PNA was virtually 100 per cent. But when the same type of tissue was maintained in Tyrode solution (containing P^{32}) under conditions favoring autolysis rather than growth, there was a 10 per cent turnover in DNA and a 60 per cent turnover in PNA.

In analyzing for nucleic acids in tissue cultures, it is current practice to separate DNA and PNA and perform phosphorus determinations on each fraction, even though other materials such as proteins may be present. The development of methods, such as that of Lepage and Heidelberger (1951) for the isolation of relatively pure nucleic acids, may be of great importance in tissue culture work, especially if micro-adaptations can be developed.

Certain types of tracers which may possibly prove superior to phosphate for evaluating nucleic acid synthesis in tissue culture are being explored in the author's laboratory. These are C^{14}-labeled purines, such as adenine, and particularly certain purine *precursors* (glycine, formic acid). The isolation of pure nucleic acids would be important for this approach.

Proteins

A protoplasmic constituent, comparable to nucleic acids in importance, is protein. Recently Gerarde, Jones, and Winnick (1952B) described a quantitative technique for measuring changes in the protein content of tissue cultures. By cultivating

relatively large quantities of embryonic chick tissues (20 to 50 mg. per tube), sufficient protein could be subsequently isolated for accurate analysis. The initial per cent of protein in a given weight of wet tissue was determined by precipitating the protein by trichloroacetic acid, and then accurately weighing the washed and dried product. This initial value (*e.g.*, 8.5 per cent for embryonic chick heart) was used to calculate the original amounts of protein present in each tube of a series, from the wet weights of tissue. By comparing the quantities of protein subsequently isolated from incubated tubes with their corresponding calculated initial values, the percentage change in protein content could be determined.

Figure 1 shows that extensive net protein synthesis occurred with embryonic chick tissues cultivated in embryo extract

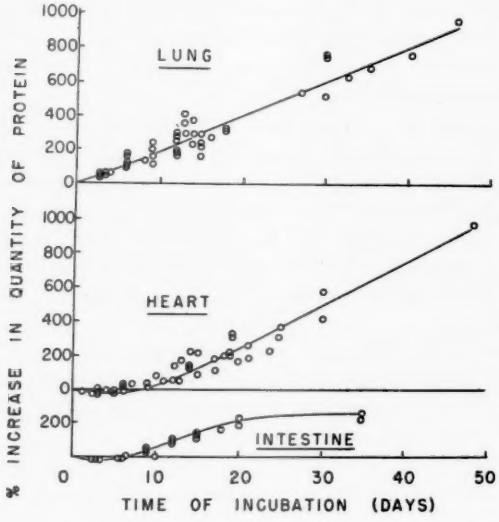

FIG. 1. Rates of protein synthesis; 12 day heart, and 14 day lung and intentine were employed. Each tube represents the pooled material from four culture tubes.

medium. In two weeks 300 per cent synthesis resulted with lung, the most rapidly growing tissue of those examined, while heart and intestine doubled their protein content.

Figure 2 illustrates the gross appearance of the roller tube cultures customarily prepared in our laboratories at the University of Iowa. The growth of the numerous tissue fragments resulted in an almost complete coverage of the inner walls of the tubes.

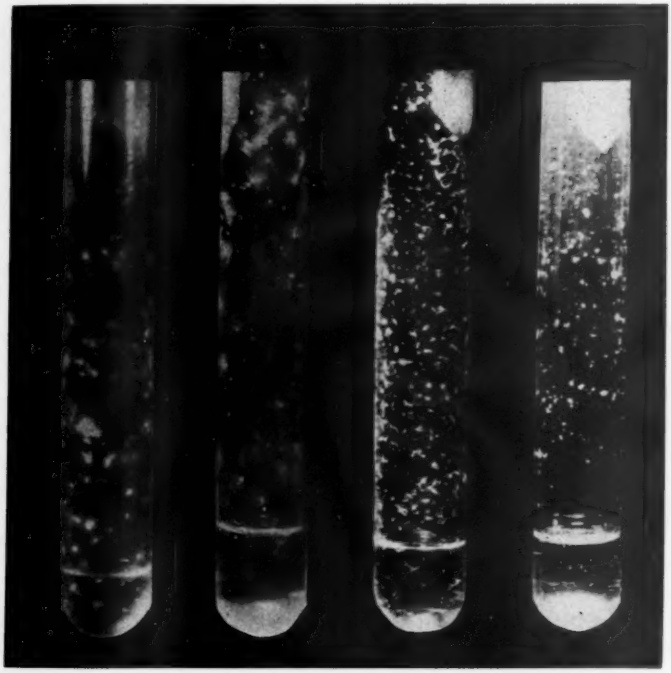

FIG. 2. Gross appearance of culture tubes containing, from left to right: (a) heart, (b) intestine, (c) lung, each after 12 days cultivation, and (d) freshly-explanted lung. (Tubes with freshly-explanted heart or intestine presented a similar appearance).

Figure 3 shows the early fibroblasts radiating from a single heart fragment.

It has been possible to maintain growth in roller tubes for more than 50 days (without subculturing). However, although the protein content continues to increase linearly with time, the

nucleic acid content decreases markedly in old cultures, with a corresponding decrease in cellular population. The available evidence suggests that the protein is largely *extra-cellular* in character and has certain properties akin to collagen. From the results to date, it appears that protein synthesis parallels cellular multiplication only during the first few days of cultivation and even during this period, may not be an ideal index of growth.

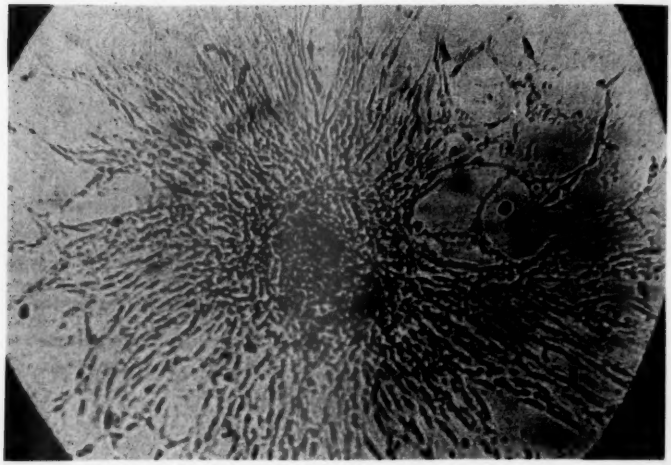

FIG. 3. Microscopic appearance of heart after 5 days. Magnification is about 30 diameters.

That the extensive increases in protein observed in the above studies are not adsorption artifacts is indicated by the fact that protein synthesis is abolished by a variety of enzyme inhibitors (azide, cyanide, fluoride, etc.) and by lowering the incubation temperature to 5°.

It may be of interest to mention that complete amino acid analyses of certain tissue culture proteins have been performed in the author's laboratory. The method was that of Moore and Stein (1949), involving chromatography on starch columns, followed by colorimetric determinations. Figure 4 gives the chromatogram obtained with protein from heart cultures. From

the peaks, the percentages of the various individual amino acids could be calculated.

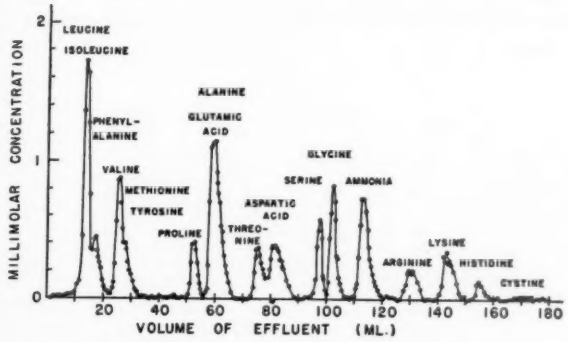

FIG. 4. Chromatographic analysis of embryonic chick heart protein, following prolonged cultivation. 4 mg. of hydrolyzed protein were employed.

Labeled Amino Acids

Many biochemists have employed isotopically-labeled amino acids in the study of protein synthesis or regeneration, using tissue slices and homogenates, as well as intact animals. Gerarde, Jones, and Winnick (1952B) found that, upon the addition of radioactive C^{14}-labeled amino acids to nutrient media, these compounds were rapidly incorporated into the proteins of tissue cultures. A surprising finding in this connection was that the greatest uptakes usually occurred, not under conditions favorable to growth, but when the tissues were maintained in suboptimal media.

Figure 5 shows that labeled glycine, alanine, and phenylalanine were most extensively utilized when present in an artificial medium containing a complete amino acid mixture (AAM). The latter contained 17 non-isotopic L-amino acids (also inorganic salts). AAM medium was inadequate for growth, and, actually, slow autolysis of the cultures occurred when it was used. Inasmuch as a turnover or replacement of amino acid residues in the proteins occurs in all cases, the uptake of labeled amino acids cannot serve as a criterion of

true protein synthesis, even under conditions of tissue growth. The finding that the turnover of one amino acid is augmented by the presence of others agrees with the results obtained by Peterson, Winnick and Greenberg (1951) with homogenates of liver tissue.

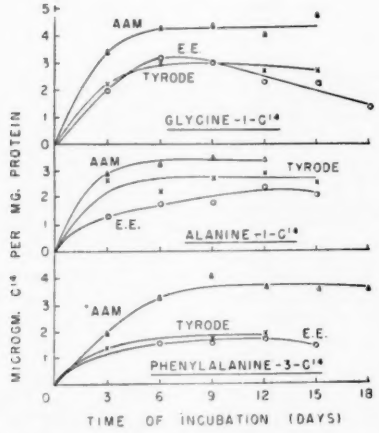

FIG. 5. Incorporation of labeled amino acids into protein of lung cultivated in different media.

In connection with the use of radioactive amino acids in tissue cultures, it has been possible to determine the exact degrees of turnover of individual amino acids in the proteins. This turnover may be expressed as the ratio of newly-incorporated (labeled) to total amino acid, for a given species such as glycine or alanine. In Figure 6 are chromatograms of hydrolyzed radioactive heart and lung proteins, derived from experiments in which a single labeled amino acid was added to the medium. The radioactivity of each chromatographic fraction has been expressed in units of amino acid concentration. It was found that, when either alanine or phenylalanine was employed in the original medium, all of the subsequent radioactivity of heart or lung protein could be accounted for by these same individual amino acids. When glycine was used,

the radioactivity was found partly in glycine and partly in serine, in agreement with the well-known metabolic interconversion of these two substances. The ratios of radioactive to total amino acids indicate degrees of replacement of the order of several per cent per day.

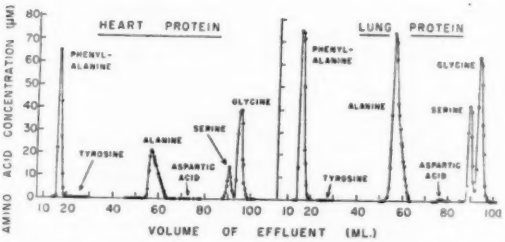

FIG. 6. Composite graphs of C^{14} distribution in tissue proteins following 2-day incubation in Tyrode solution containing a radioactive amino acid. Each labeled compound was used in a separate experiment.

A somewhat different way of measuring this dynamic activity of tissue proteins is to first render the proteins of a given culture radioactive by the presence of a C^{14}-labeled amino acid in the nutrient medium, and to then *withhold* the labeled compound from the medium, and observe the rate of *release* of radioactivity from the proteins. Such experiments, performed by Gerarde, Jones, and Winnick (1952B), have yielded "half life" values for certain amino acids in embryonic tissue proteins. Illustrative data for C^{14}-labeled glycine is shown in Figure 7. This amino acid was present in the 25 per cent embryo extract medium for the first 12 days. During this time the mass of protein increased in the customary manner (lower graph) and C^{14} accumulated in the proteins (upper graphs). Then the medium was replaced with a 5 per cent embryo extract, inadequate to support further protein synthesis, and containing no glycine. Examination of the upper right-hand portion of Figure 7 shows that intestine lost half its C^{14} between the 15th and 24th day. This indicates a half-life of 9 days. The corresponding half-lives of glycine in heart and lung proteins were 6 and 7 days, respectively. From these values

it appears that intestine, lung, and heart proteins renewed approximately 5.5, 7, and 8.5 per cent of their glycine residues per day. These values are rather similar to those reported by other investigators for *in vivo* experiments with rats.

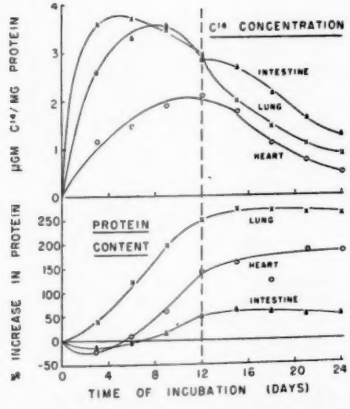

FIG. 7. Incorporation of labeled glycine, and subsequent release from tissue culture proteins.

Role of Biological Nutrient Media

None of the artificial media employed thus far appear to even approach biological extracts, with respect to growth-promoting properties. Among the most challenging of the natural nutrients are embryonic extracts. Our knowledge of the exact roles of even the major components in these juices is quite fragmentary. However, the pioneer studies of Fischer are full of suggestions for further work. Chemical fractionation leading to the assay of isolated proteins and nucleic acids and their digestion products are indicated. Dialysis and ultrafiltration techniques may be expected to throw light on the parts played by diffusible metabolites. Fractional centrifugation and even chromatography may conceivably be employed in examining biological nutrients. An ultimate goal here is, of course, the

determination of the exact nutritional requirements of specific types of cells.

A related question is that concerned with the levels of complexity at which metabolites are utilized by tissue cultures during growth. For example, the source of tissue proteins may be the simple amino acids in biological extracts. But it is also possible that the cells may absorb and utilize peptides released from partially digested proteins in the medium. It is even conceivable that whole protein molecules may be assimilated, with only minor structural modifications. Similarly, in the case of nucleic acid synthesis, the cells may absorb and utilize free purines and pyrimidines (or their precursors). They may utilize these compounds at the nucleoside or nucleotide levels. Or, possible, polynucleotides of the medium may be built into cellular structures without extensive degradation.

One way of studying such problems is through the use of isotopically-labeled proteins and nucleic acids, in which the tagged amino acids or purines and pyrimidines are in *bound form.* Such substances have been prepared at Iowa by injecting suitable labeled compounds into 2-day-fertile eggs, and eight days later preparing embryo extracts. For instance, if labeled phenylalanine is employed, the subsequent radioactivity of the embryo extract is found to be solely due to protein-bound phenylalanine. Experiments can then be performed to determine whether or not the utilization of this amino acid by tissues requires its prior release as free phenylalanine from the embryo extract proteins.

The biosynthesis of complex substances, such as proteins and nucleic acids, cannot be divorced from the intermediary metabolism of these materials. In view of the current great interest in the pathways of synthesis, interconversion, and degradation of amino acids, purines, pyrimidines, lipids, and carbohydrates, it would seem a natural development to study these problems in cultures of various normal and cancerous tissues, under carefully controlled conditions. The numerous enzymic systems, their associated cofactors, and even the mode of action of hormones, are likely to receive increasing attention in tissue culture research in future years.

REFERENCES

Boivin, A., Vendrely, R., and Vendrely, C.: L'acide désoxyribonucléique du noyau cellulaire, dépositaire des caractères hérèditaries; arguments d'ordre analytique. *Compt. rendu*, 226:1061–63, 1948.

Davidson, J. N., Leslie I., and Waymouth, C.: Nucleoprotein content of fibroblasts growing in vitro. 4. Changes in ribonucleic and desoxyribonucleic acid phosphorus content. *Biochem. J.*, 44:5–17, 1949.

Gerarde, H. W., Jones, M., and Winnick, T.: Nucleic acid synthesis and turnover in embryonic chick tissue cultures, *J. Biol. Chem.* 196, 69 1952A.

Quantitative measurement of protein synthesis and amino acid turnover in chick embryonic tissue cultures. *J. Biol. Chem.* 196, 51, 1952B.

Hull, W., and Kirk, P. L.: Tissue culture studies. II. Relationship of nucleic acid increases to growth of cells in vitro. *J. Gen. Physiol.*, 33:327–334, 1950A.

Tissue culture studies. III. Relationship of incorporation of radioactive phosphorus in nucleic acid to growth of cells in vitro. *J. Gen. Physiol.*, 33:335–342, 1950B.

LePage, G. A., and Heidelberger, C., Incorporation of glycine-2-C^{14} into proteins and nucleic acids of the rat. *J. Biol. Chem.*, 188:593–602, 1951.

Moore, S., and Stein, W. H., Chromatography of amino acids on starch columns. Solvent mixtures for the fractionation of protein hydrolysates. *J. Biol. Chem.*, 178:53–77, 1949.

Peterson, E. A., Winnick, T., and Greenberg, D. M., Stimulation by the adenylic acid system of amino acid incorporation into protein of liver graanules. *J. Am. Chem. Soc.*, 73:503–504, 1951.

SUR UNE METHODE DE CULTURE D'ORGANES EMBRYONNAIRES "IN VITRO"*

ETIENNE WOLFF ET KATY HAFFEN

Nous avons cherché à obtenir le développement in vitro des gonades de l'embryon d'Oiseau, explantées avant le stade de la différenciation sexuelle. Après avoir essayé les techniques de culture utilisées par Strangeways et Fell (1926), Fell (1928), Martinovitch (1938), Gaillard (1949–50), nous avons été amenés à adopter une technique nouvelle qui s'est révélée favorable à la culture de nombreux organes embryonnaires.[1] Nous avons remarqué que la plupart des milieux généralement employés dans la culture de tissus sont trop riches en éléments nutritifs pour des organes aussi délicats que les gonades indifférenciées. Ils favorisent la pullulation incohérente d'éléments cellulaires qui migrent hors de l'organe, le désagrègent et l'étouffent. La "culture de tissus," telle qu'elle est habituellement pratiquée, est aussi nuisible à la "*culture d'organes*" que l'infection bactérienne. C'est en diminuant la teneur en substances nutritives du milieu qu'on assure la survie et le développement de l'organe. Nous avons cherché d'autre part à obtenir un substratum constant qui assure à l'explant des conditions favorables de respiration, d'absorption et d'humidité, à l'image du milieu où vivent de petits organismes terrestres des lieux humides, dépourvus d'appareil respiratoire et circulatoire.

Nous avons adopté pour la plupart des expériences le milieu de composition suivante:

MILIEU I:

Gélose contenant lgr. d'agar
pour 100 cc de liquide de Gey .....6 parties
Extrait d'embryons de poulet de 7 jours
dilué à 50 p. 100 dans le liquide de

*From the Laboratoire d'Embryologie expérimentale de la Faculté des Sciences, Strasbourg, France. Received for publication December 15, 1951.

(1)Nous tenons à adresser nos vifs remerciements au Dr Ch. Pomerat, Professeur de Cytologie à l'Université de Texas, qui a fait profiter l'un de nous, au cours d'un séjour dans son laboratoire, de sa grande expérience des cultures de tissus, et dont les suggestions ont servi de point de départ à nos essais.

Tyrode ..... 3 parties
Liquide de Tyrode ..... 3 parties
S Pénicilline retard (100 à 200 U. Oxford) ..... 1 goutte

Une variante de ce milieu utilisé dans certaines expériences a été la suivante.

MILIEU II:

Gélose (même constitution) ..... 6 parties
Extrait d'embryons de poulet de 7 jours dilué à 50% ..... 2 parties
Serum de coq ou de cheval ..... 1 partie
Liquide de Tyrode ..... 3 parties
S Pénicilline retard ..... 1 goutte

D'autres variantes ont été utilisées, dans lesquelles les proportions de gélose restent inchangées, et où seules varient les proportions de jus d'embryons, de serum, éventuellement de liquide de Tyrode. Mais de tels milieux conviennent surtout à des organes plus âgés et plus massifs.

Les récipients de culture sont des verres de montre du type "salières," à fond plat et à cavité hémisphèreque, déjà préconisés par Gaillard (1949). Un couvercle de verre carré peut s'adapter exactement sur la face supérieure du récipient.

Le milieu est préparé de la manière suivante: la gélose chauffée au bain-marie à la température de 70 à 80°C est versée au compte gouttes dans le récipient; on ajoute 3 parties de Tyrode, puis 3 parties des liquides nutritifs, enfin une goutte de pénicilline.[1] On mélange intimement ces constituants en agitant avec une baguette de verre; on laisse le milieu se refroidir et se solidifier. La gelée obtenue doit être élastique et parfaitement homogène.

L'organe à explanter est disséqué sous un microscope binoculaire de type Zeiss-Opton. Cet instrument dont les objectifs tournent dans une boîte entièrement close, permet de changer de grossissement pendant l'opération sans risquer d'infecter l'embryon ou le milieu de culture. L'organe est découpé à l'aide d'aiguilles de verre, de fins couteaux à iridectomie et de ciseaux

(1) Tous les liquides, à l'exception de la gélose, sont à la température de la chambre de culture. Il importe de verser le Tyrode avant le jus d'embryon et le serum, afin qu'ils ne soient pas dénaturés par la chaleur.

de Pascheff. Il est transporté à l'aide d'une cuiller à transplantation sur le milieu de culture, où il est déposé avec précaution. Il faut se garder d'écorcher la surface de la gélose, qui doit rester lisse et homogène, et sur laquelle l'explant doit être posé avec précautions. Il ne doit pas être placé dans une crevasse du milieu, où il risque d'être noyé dans le liquide exsudé par la gélose. Il arrive que l'organe déprime la surface et s'enfonce légèrement dans la gelée. Cette circonstance n'est pas défavorable à la culture, car l'explant continue a être en contact avec l'atmosphère par sa face supérieure. Il ne doit jamais être complètement enrobé dans la gélose. Cet accident ne se produit pas quand le milieu est préparé exactement dons les conditions qui viennent d'être décrites: la consistance du milieu est alors invariable et se prête bien à l'explanation.

L'explant est débarrassé des gouttes de liquide qui ont été déposées avec lui surla surface du milieu. Elles sont aspirees par de petits morceaux de papier filtre, sous le contrôle du binoculaire. Il importe que le liquide soit complètement épuisé. L'humidité du substratum et l'atmosphère saturée du récipient suffisent à empêcher la dessication de l'explant. Celui-ci respire l'air atmosphérique par la plus grande partie de sa surface, tandis qu'il absorbe le liquide nutritif dans la région du hile qu'on a tournée vers le milieu de culture.

On lute alors le couvercle du récipient à la paraffine. Aucune fente ne doit subsister entre le couvercle et la salière. Les récipients de culture sont placés dans une étuve à la température de 38°.

Toutes les opérations sont faites avec les précautions rigoureuses d'asepsie habituelles aux cultures de tissus: chambre de culture, prélèvements aseptiques, récipients et instruments stériles. Il est recommandé aux expérimentateurs, généralement au nombre de deux, de se couvrir le visage d'un masque de gaze.

On remarque que, dans le milieu I, qui a été le plus souvent adopté après de nombreux essais, le seul élément nutritif est l'extrait d'embryon, qui sert à la fois d'ailment et de substance stimulatrice du développement. Les organes explantés, par exemple les gonades, ferment bientôt leurs blessures en s'entourant complètement d'une fine membrane de nature épithéliale. Cette "épithélisation," comme l'a déjà remarqué Gaillard

(1950), est une condition indispensable à la survie et au développement de l'organe. Dans quelques cas, la région du hile reste ouverte, et les tissus poussent à ce niveau des prolongements dans le milieu qu'ils entuorent et cloisonnent.

Le milieu fondamental dont nous préconisons l'emploi offre l'avantage d'un substratum solide de constitution invariable et de propriétés physiques constantes: la gélose, préparée toujours dans les mêmes conditions et mélangée à température constante à un égal volume de liquide isotonique au plasma sanguin (extrait d'embryon et liquide de Tyrode), offre aux explants un substratum ayant une consistance, une élasticité, une humidité très voisines d'une expérience à l'autre. De très légers écarts dans les proportions des constituants énumérés ci-dessus suffisent à faire échouer complètement les cultures.

Les explants sont lavés et repiqués aussi souvent qu'il convient, en général tous les deux jours, parfois plus souvent. L'èxsudation de liquide, la formation d'une légère auréole de culture autour de l'organe sont les signes qui imposent cette intervention. Les organes sont lavés sur place dans un léger courrant de liquide de Tyrode versé avec une fine pipette; ils sont débarrassés, s'il y a lieu, des petits amas de culture cellulaire qui les entourent, puis déplacés et fixés en un autre point de la surface du milieu. Des cultures de 5 à 7 jours doivent être repiquées sur milieu frais.

Cette technique a déjà été appliquée à la culture de différents organes embryonnaires et à la solution de plusieurs problèmes. Elle s'est révélée une technique de choix pour la culture de gonades différenciées ou indifférenciées de l'embryon de canard et de poulet (Et. Wolff et K. Haffen 1951). Les gonades de canard, prélevées après le stade de la différenciation sexuelle, poursuivent leur développement dans le sens mâle ou femelle. Prélevées avant le stade de la différenciation, elles se différencient in vitro en ovaires ou en testicules, conformément à leur sexe génétique. Leur aspect extérieur est caractèristique aussi bien que leur structure histologique. Un ovaire en voie de différenciation est immédiatement reconnaissable à la frange plate et transparente qui traduit extérieurement la prolifération corticale (fig. 1). Un testicule a l'aspect d'un boyau opaque, renflé, grossièrement cylindrique (fig. 2), entouré d'un épithélium

mince et continu. Nous avons réalisé des parabioses entre gonades indifférenciées. Les explants se soudent intimement par leurs faces en contact, se fusionnent plus ou moins en se développant (fig. 3 et 4). Quand une gonade de sexe génétique femelle est unie à une gonade de sexe génétique mâle, la première se différencie en ovaire, la seconde édifie une glande intersexuée de structure très analogue à un ovaire (fig. 5). Si l'on ajoute au milieu une hormone femelle cristallisée, les gonades génétiquement mâles sont féminisées comme par l'hormone femelle émanée d'un ovaire (Et. Wolff et K. Haffen 1951).

FIGURE 1 FIGURE 2

FIG. 1. Ovaire d'embryon de Canard prélevé au 9e jour de l'incubation et cultivé pendant 5 jours *in vitro*. On remarque une frange plate et transparente correspondant à la prolifération corticale. G: 40.

FIG. 2. Testicule de poulet prélevé au 9e jour et cultivé pendant 3 jours *in vitro*. G: 40.

D'autres organes à différenciation sexuelle précoce ont été soumis à l'épreuve de la culture. Des syrinx d'embryons de Canard sont explantées avant le 9ème jour d'incubation, au stade où elles sont encore sexuellement indifférenciées. Quel que soit leur sexe génétique, toutes se différencient suivant le type mâle (Et. Wolff Em. Wolff et K. Haffen 1951): les anneaux cartilagineux se différencient in vitro en présentant l'asymétrie caractéristique d'unne syrinx mâle. Au contraire, s'i les syrinx sont

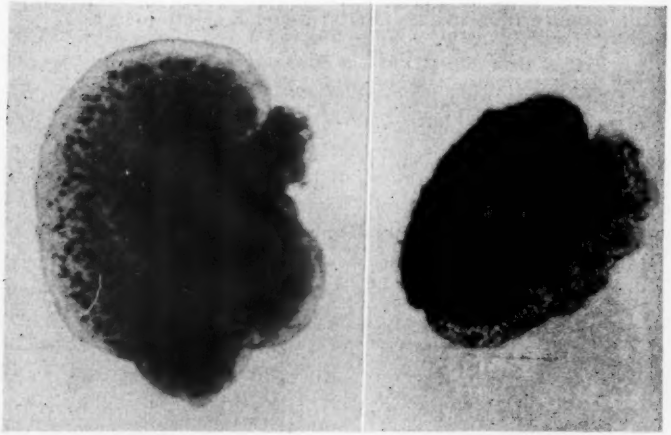

FIGURE 3 FIGURE 4

FIG. 3. Parabiose de 2 gonades de Canard prélevées au stade indifférencié, et cultivées 4 jours. L'une, génétiquement femelle, se différencie en ovaire; l'autre génétiquement mâle, entourée par la première, se différencie en une glande intersevuée, d'aspect voisin d'un ovaire. G. 55.

FIG. 4. Association d'une gonade droite et d'une gonade gauche de Canard, prélevées sur deux embryons différents au 9e jour de l'incubation et cultivées *in vitro* pendant 5 jours. La gonade gauche se différencie en ovaire, la gonade droite en testicule. G: 30.

prélevées après la différenciation sexuelle des gonades, elles se développent suivant le type sexuel des gonades. Si l'on cultive les syrinx indifférenciées sur un milieu contenant une hormone femelle cristallisée, elles se différencient suivant le type femelle, caractérisé par des anneaux cartilagineux grêles et symétriques (Et. Wolff et Em. Wolff 1951). Ces résultats démontrent que le type mâle correspond au type neutre ou asexué. C'est l'

hormone femelle de la gonade en la substance oestrogène cristallisée qui provoque la différenciation femelle.

De même, le tubercule génital de l'embryon de canard a été explanté avant et après le début de la différenciation sexuelle (Et. Wolff et Em. Wolff 1951). Les résultats ont conduit aux mêmes conclusions que les expériences sur la syrinx: la forme mâle, tordue en hélice, se développe en l'absence d'hormone, la forme femelle, rudimentaire et non enroulée, est seule conditionnée par une hormone.

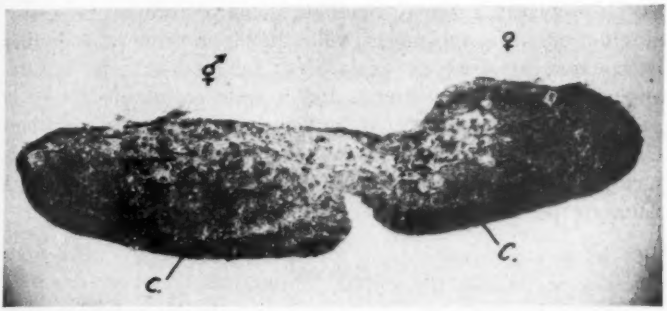

FIG. 5. Coupe transversale à travers deux gonades gauches associées au stade indifférent (8e jour d'incubation). A droite sur la figure, différenciation ovarienne; à gauche formation d'une glande intersexuée à partir d'une ébauche génétiquement mâle. Durée de la culture: 6 jours. *C.*: cortex ovarien. G: 11o.

D'autres organes embryonnaires ont été éprouvés en cultures. Les canaux de Müller entiers, des fragments de mesonephros des embryons de poulet et de canard, les vésicules optiques des Oiseaux ont été cultivées avec succès. Il convient d'ajouter à cette première liste les gonades de souris mâles et femelles, prélevées sur des embryons de 11 à 15 jours, et explantées soit séparément soit en parabiose avec des gonades d'autres embryons, elles ont donné d'evcellents développements en culture.

Ces premiers résultats permettent de se rendre compte des possibilités et des promesses d'une méthode simple qui a l'avantage de fournir un substrat de propriétés constantes. Seules peuvent être modifiées les proportions relatives des éléments nutritifs et des liquides physiologiques. L'âge des embryons qui

fournissent l'extrait a une influence sur le développement de certains tissus, ainsi que l'a observé Miszurski (1939) et comme nous l'avons constaté nous-mêmes.

Nous insisterons en terminant sur les possibilités expérimentales qu'a montrées cette nouvelle méthode. Elle permet d'une part d'analyser les potentialités évolutives d'un organe explanté à un stade déterminé de son développement. Elle permet d'autre part d'associer deux organes provenant du même embryon ou d'un autre organisme, d'étudier leurs réactions mutuelles, en mettant tout le reste de l'organisme hors circuit. De plus, on peut aisément modifier la composition du milieu en ajoutant certaines substances, telles que des hormones, et étudier leur action sur l'organe explanté et les modifications qu'elles apportent au développement. Enfin cette technique donne la possibilité de recueillir et d'étudier directement les sécrétions d'un explant. Nous pensons qu'elle pourra rendre de grands services à l'organogénèse expérimentale et plus généralement à l'analyse physiologique.

Résumé

Les techniques habituelles de cultures de tissus se sont révélées impropres à la culture des gonades embryonnaires.

Les conditions favorables au maintien de l'intégrité et au développement d'un organe isolé in vitro sont réalisées grâce aux qualités physiques et nutritives du milieu dont nous préconisons l'emploi. Les milieux trop riches en éléments nutritifs favorisent une prolifération intense des cellules hors de l'organe qui se trouve rapidement dissocié.

C'est un milieu relativement pauvre en aliments qui nous a donné les meilleurs résultats.

De la gélose préparée toujours dans les mêmes conditions et mélangée à un volume égal de liquide physiologique constitue le substratum solide de constitution invariable et de propriétés physiques constantes.

L'extrait d'embryon dilué à 50% se révèle suffisant pour servir à la fois d'aliment et de substance stimulatrice.

Toutefois il est possible de remplacer une ou deux parties de jus d'embryon ou de Tyrode par une quantité correspondante de

serum de coq ou de cheval. Mais les moindres écarts dans les proportions des constituants doivent être évités.

Le milieu est préparé des verres de montre du type "salières."

Les explants sont déposés sur la surface du milieu coagulé et débarrassés des gouttes de liquide entraînées au cours de la manipulation. La surface de la gélose doit rester lisse et homogène.

Les explants sont lavés et repiqués en général tous les 2 jours. Cette technique a déjà été appliquée à la culture de différents organes embryonnaires et à la solution de plusieurs problèmes. Elle a permis de réaliser la différenciation normale des gonades suivant leur sexe génétique et l'intersexualité des gonades de l'embryon de canard dans différentes conditions expérimentales (parabiose directe et addition d'hormone au milieu).

L'explantation des syrinx d'embryons de canard a donné des résultats qui démontrent que le type mâle correspond au type neutre ou asexué et que seule la présence d'hormone femelle provoque la différenciation femelle.

D'autre part des canaux de Müller, des fragments de mésonéphros, des vésicules optiques d'embryons d'oiseaux, des gonades embryonnaires de souris mâles et femelles ont été cultivées avec succès.

REFERENCES

Fell, H. B.: The development in vitro of the isolated otocyst of the embryonic fowl. *Arch. f. exp. Zellforsch.*, 7:69, 1928.

Gaillard, P. J.: Germinal or covering epithelium. 3° *Journ. cyto-embryol.* belgo-neerland., :4–9, 1949.

Gaillard, P. J.: Sex cell formation in explants of the foetal human ovarian cortex. I et II. *Korr. Ned. Akad. v. Weteusch.* 53:1300–1347, 1950.

Martinovitch, P. N.: The development in vitro of the Mammalian gonad. Ovary and ovogenesis. *Proc. Roy. Soc. ser. B*, 125:232–249, 1938.

Miszurski, B.: Recherches sur l'influence des extraits des embryons de differents âge sur la croissance et la différenciation du cartilage et de l'os en culture. *Arch. f. exp. Zellforsch.*, 22:130–131, 1939.

Strangeways, T. S. P., et Fell, H. B.: Experimental studies on the differentiation of embryonic tissues growing in vivo and in vitro. I. The development of the undifferentiated limb bud. *Proc. Roy. Soc. ser. B.*, 99: 340–364, 1926.

Wolff, Et. et Haffen, K.: Sur la différenciation in vitro des gonades embryonnaire des oiseaux. *C. R. Soc. Biol.* scéance du 6 juillet, 1951.

Wolff, Et. et Haffen, K.: Sur la culture in vitro des glandes génitales des embryons d'oiseaux. Obtention de la différenciation sexuelle normale et de l'intersexualité experimentale des gonades explantés. *C. R. Acad. Sc.*, 233:439–441, 1951.

Wolff, Et. et Wolff, Em.: Action de l'hormone femelle sur la différenciation sexuelle de la syrinx de canard cultivée in vitro. *C. R. Soc. de Biol.* scéance du 17 novembre., 1951.

Wolff, Et., Wolf, Em., et Haffen, K.: Sur la différenciation in vitro de la syrinx chez l'embryon de canard. *C. R. Acad. Sc.*, 233:500–502, 1951.

CALLING ATTENTION TO:

Items of Possible Interest to Our Friends

January, 1952

New Year Seriousness: A Castiglioni recalls value of doctrine of contagium vivum of G Fracastoro (1484–1553) (*Sci Med Ital* 1/747/51). RB Cattell suggests concepts for measuring leadership (*Human Rel* 4/161/51). Sir Norwood East analyses *Society & the Criminal* (CC Thomas, Springfield, Ill, 51, 452pp, $8.5). H Fischer edits historical festschrift for HE Eigerist (*Gesnerus* 8/1–194/51). JF Fulton joins *The Great Medical Bibliographers* (Univ Pennsylvania Press, Phila, 51, 122p, 37figs, $4). RM Green translates *Galen's Hygiene* (CC Thomas, Springfield, Ill, 51, 304pp, $5.75). M Ploys edits finely illustrated memorial tribute to alkaliodists J Pelletier (1788–1842) & JB Caventou (1795–1877) (*Rev Palud Med Trop* Oct 51, 107pp). H Reichenbach greets *The Rise of Scientific Philosophy* (Univ California Press, Berkeley, 51, 333pp, $3.75). EW Teale delightfully describes *North with the Spring* (Dodd Mead, NY, 51, 366pp, $5). E Trueblood cautions about *The Life We Prize* (Harpers, NY, 51, 218pp, $2.5). H Williams biographizes in *The Healing Touch* (CC Thomas, Springfield, Ill, 51, 408pp $7).

Preclinical: AD Bergner & SH Durlacher find diminished CuS deposition at motor endplates in anticholinesterase poisoning (*Am J Path* 27/1011/51). P Constantinides finds mitotic growth of tubule cells of enlarged kidney following adrenal enlargement in 20 hrs acute stress (*Endocrin* 49/512/51). GM Findlay says viruses are nucleoproteins like those of cells which they specifically parasitize & result from cell mutations (*J Rov Microse Soc* Series III, 71/151/51). A Goldstein indicates significance of kinetics of competitive inhibition of cholinesterase (*Arch Biochem Biophys* 34/169/51). RA Hughes & F Robinson review neurochemical mechanisms involving acetylcholine & central nervous system (*Yale J Biol Med* 24/35/51). JM Jensen & DE Clark find thyroxin localizes in neurohypophysis but not iodine (*J Lab Clin Med* 38/663/51). P Lacroix & MJ Dallenague consider mechanisms of bone formation (*J Physiologie* 43/385, 425/51). A Lipschutz analyses evolution of tumor cells (*Rev Fac Hum Sci Montevideo* 6/183/50; *Rev Canad Biol* 10/341/51). GB Mider reviews nitrogen & energy metabolism in cancerous subjects (*Cancer Res* 11/821/51). E Odeblad shows high P^{32} in developing corpora lutea, low in retrogression, & high concentration in granulosa cells in growing follicles (*Acta Obs Gyn Scand* 31/63/51). I Pataky & Co find creatinine inhibits metabolism-increasing effect of thyroid (*Acta Physiol Acad Sci Hung* 2/199/51). JP Peters analyses inter-relationships of foodstuffs in metabolism (*Yale J Biol Med* 24/48/51). K Winterfeld surveys lupin alkaloids (*Arzneiforsch* 1/205–51). A Wollenberger says cardiac glycosides help contractile

system of myocardium use adenosine triphosphate (*J Pharmacol* 103/123/51). HF Zipf & H Philipsborn develop quantitative methods for studying drug combinations (*Arsneiforsch* 1/199/51).

CLINICAL: A Ahto shows high hereditary & environmental influence in dangerous habitual criminals (*Suppl 69, Acta Psych Neurol*, Helsinki 51, 168pp). J Armin & RT Grant say gross pulmonary fat embolism in injured men unlikely to cause symptoms or death (*Clin Sci* 10/441/51). D Bovet & VG Longo discuss antinicotine drugs in treating Parkinsonism (*J Pharmacol* 102/22/51). EGL Bywaters & Co think there is altered hyaluronic acid metabolism in rheumatic arthritis & fever (*Brit Med J* 2/1178/Nov 17/51). L Fleck & Co continue extensive studies on leukergy, indicating diagnostic value (*Ann Univ M Curie-Sklodowska*, Ser D, 5/343–537/50). H Garland & MJ Parsonage condemn use of bromides (*Univ Leeds Med Mag* 21/61/51). DB Jones finds insterstitial edema characteristic of glomerular lesion of eclampsia (*Am J Path* 27/991/51). H Kalliala & MJ Karvonen find release of post-pituitary during suckling in lactating women (*Ann Med Exp Biol Fenniae* 29/233/51). JC Lopez reviews antimycotic chemotherapy (*Clin Lab: Rev Esp Cien Med* 52/179/51). KS Mathur & PW Waki show no fixed pattern of hepatic fibrosis in cardiac decompensation (*Indian J Med Sci* 5/151/51). HHW Miles & S Cobb criticize idea of neurocirculatory asthenia (*New Eng J Med* 245/711/Nov 8/51). JR O'Brien concludes bleeding time estimations are no more informative than platelet counts (*J Clin Path* 4/272/51). GW Pickering finds vascular spasm uncommon (*Lancet* 2/845/Nov 10/51). LH Schmidt & WL Squires find cortisone intensifies malarial symptoms (*J Exp Med* 94/501/51). RL Swank & V Nilmot show heparin IV clears lipemic plasma & that chylmicra are 97% neutral fat & 3% cholesterol (*Am J Physiol* 167/403/51). M Tschudin says average insulin coma dose is 2.2–2.4 units per kilo body weight independent of sex, age or constitution (*Conf Neurol* 11/354/51). JB Villasenor & Co show prognostic value of hemoglobin levels in portal cirrhosis (*Rev Invest Clin* 3/309–51). H Wick recommends butyl scopolammonium Br ("Buscopan") as safe powerful spasmolytic and antinicotinic (*Arch Exp Path Pharmakol* 213/485/51).

FEBRUARY, 1952

HISTORICAL: GW Adams examines *Doctors in Blue: the Medical History of the Union Army in the Civil War* (Schuman, NY, 52, 256pp, $4). A & NL Clow describe *The Chemical Revolution* (Batchworth Press, Lond, 52, 712pp, $11). D Donovan & D Whitney tell fascinating story of Painless Parker, Last of America's Tooth-Plumbers (*Collier's* Jan 2, 9 & 16, 52). LI Dublin really offers *The Facts of Life from Birth to Death* (Macmillan, NY, 52, 460pp, $5). W Ebbecke offers appreciation of Johannes Muller (1801–58) (Schmorl, Hannover, 51, 191pp, 9DM). TVH Ellinger translates *Hippocrates on Intercourse & Pregnancy* (Schuman, NY, 52, 128pp, $2.5). JN Friend surveys *Man & the Chemical Elements* (Griffin,

Lond, 52, 352pp, 27s6d). EF Horine edits D Drake's famous 1820 *Inaugural Discourse on Medical Education* (Schuman, NY, 52, 100pp, $5). HA Kemp calls for study of medical sociology (*South Med J* 44/1070/51). ET Martin euogizes *Thomas Jefferson, Scientist* (Schuman, NY, 52, 300 pp, $4). C Nieder anthologizes *Great Shipwrecks & Castaways* (Harper, NY, 52, 238pp, $3). JG Neihardt records Sioux culture in *When the Tree Flowered* (Macmillan, NY, 52, 320pp, $4). PB Rehberg eulogizes A Krogh (1874–1949) (*Yale J Biol Med* 24/83/51). JT Shotwell describes the faith of an historian (*Sat Rev*, Dec 29, 51). HE Sigerist wisely tells how he lives with hypertension (*Atlantic* 189/25/52). For a brilliant appreciation of current Mexican art see V Stewart's *45 Contemporary Mexican Artists: A Twentieth Century Renaissance* (Stanford Univ Press, 52, 167pp, $10). FG Young discusses Thomas Addison (1793–1860) & cortisone (*Brit Med J* 2/1535/Dec 29/51). JZ Young offers *A Biologist's Reflection on the Brain* (Oxford Univ Press, Lond, 51, 176pp. 7s6d).

Clinical: GS Berenson & GE Burch show cardiac patients are hurt by hot humid environment (*Am J Med Sci* 223/45/52). G deTakats & MH Marshall find post-operative stress disturbs blood clotting equilibrium (*Surg* 31/13/52). AP Friedman & Co review migraine (*Post-Grad Med* 11/33/52). HE Hoff & CG Breckenridge describe levels of integration of 3 basic respiratory patterns of eupnea, sighing & gasping (*J Neurophysiol* 15/47/52). E Kaplan & Co show inherited Hb abnormality related to new type of sickle-cell anemia (*Blood* 6/1240/51). WSS Ladell notes regression in sweating as index of acclimatization to heat & humidity (*J Physiol* 115/296/51). JR Lewis describes pyridyl-ethyl-phthalimide as potent safe analgesic (*Arch Internat Pharmaco* 88/142/51). WS McClellan reviews *Physical Medicine & Hehabilitation for the Aged* (CC Thomas, Springfield, Ill, 51, 81pp, $2). MP Margolis surveys sicklecell anemia (*Med* 30/357/51). SH Rinzler monographs *Cardiac Pain* (CC Thomas, Springfield, Ill, 51, 140pp, $2.5). PE Spangler recommends casein & lauryl sulfate gel dressing with coarse zinc acetate gauze for severe burns (*US Armed for Med J* 3/105/52). H Waelsch & Co find 1-glutamic acid improves mental performance of mental defectives (*J Nerv Ment Dis* 114/491/51). GE Wakerlin reviews kidney & hypertension (*J Urol* 67/27/52).

Pre-Clinical: TK Adler & FH Shaw find enzyme liberation of morphine fr codeine (*J Pharmacol* 104/1/52). HG Cassidy discusses *Adsorption & Chromatography* (Interscience, NY, 51, 360pp, $6). E Cerkovnikov & Co describe cellular chemical sex differences with methylene blue reactions (*Izv Izd Farmakol Inst Zagreb* 6/1/51). P Fatt & B Katz record motor endplate potentials with intracellular electrodes noting fall in membrane resistance during muscle spike (*J Physiol* 115/320/51). TAB Harris considers *Mode of Action of Anesthetics* (Livingstone, Edinburgh, 51, 780pp, 42s). G Liljestrand shows acetylcholine is chemical mediator in carotid body (*Acta Physiol Scand* 24/225/51). J Mardones confirms relation between B vitamin deficiency & alcohol intake in rats (*Quart J Stud Alc* 12/563/51). FS Philips & Co show nephrotoxic action of adenine

(*J Pharmacol* 104/20/52). FH Pollard & JFW McOrmie describe analysis of inorganic cmpds by paper chromatography (*Endeavor* 10/213/51). JT Randall reports physical studies on cellular systems (*Proc Roy Soc B.*, 138/301/51). Our HG Swann & Co describe intrarenal venous pressure mechanisms (*Science* 115/64/Jan 18/52). DW Woolley pioneers *A Study of Antimetabolites* (Wiley, NY, 52, 269pp, $5).

COLLECTOR'S ITEM: W Reich, M.D., authority on sex, cancer, character, metaphysics, orgone energy accumulator, and publisher of *Orgone Energy Bulletin* for Orgone Institute Press of Rangeley, Maine.

MARCH, 1952

PRECLINICAL: W Andrew describes *Cellular Changes with Age* (CC Thomas, Springfield, Ill, 52, 74pp, $2.5). R Barer outlines combined phase-contrast & interference contrast microscopy (*Nature* 169/108/Jan 19/52). PR Cannon & Co note importance of potassium in tissue protein synthesis (*Metab Clin Exp* 1/49/52). DJ Finney analyses precision of biological assay (*Acta Pharmacol Tox* 8/55/52). GB Forbes describes chemical growth in man regarding Na, K & H_2O conforming to differential growth equation y equals bx^k (*Pediatrics* 9/58/52). JBJ Glass & Co note relation of glandular mucoprotein to intrinsic anti-anemia factor (*Science* 115/101/Feb 1/52). CA Hogben proposes anion exchange as mechanism of gastric HCl formation (*Proc Nat Acad Sci* 38/13/52). RH Houssay discusses adrenals in hypoxia & histamine poisoning (Ateneo, Buenos Aires, 174pp, 51, $2). RW Miner edits symposium on influence of hormones on enzymes (*Ann NY Acad Sci* 54/531–728/Dec 28/51). T Poulson studies CO_2 anesthesia (*Acta Pharmacol Tox* 8/30/52). E Stangl reviews data on vitamin F unsaturated fatty acid (*Internat Rev Vit Res* (23/164/51). JF Tait & Co indicate new minerally active agent in adrenal extract (*Lancet* 1/122/Jan 19/52). JM Tarmer finds mean serum cholesterol 190 mg/100 ml (sd 28) with correlation with endomorphic component of physique (*J Physiol* 115/371/51). R Wagner & E Kapal analyze elastic properties of aorta (*Klin Wchnschr* 30/1/Jan 1/52).

IN GENERAL: IS Bengelsdorf translates DN Kursanov & Co's item (*Usp Khim* 9/529/50) showing how political line in Sovietland controls scientific theory (*J Chem Educ* 29/2/52). MT Copeland observes *The Executive at Work* (Harvard Univ Press, Cambridge, 51, 278pp. $3.75). R Flesch describes *The Art of Clear Thinking* (Harper, NY, 51, 223pp, $2.75). HJL Marriott notes *Medical Milestones* (Wms & Wilkins, Balt, 52, 305pp, $3.5). TR McKeldin & JC Krants Jr describe *The Art of Eloquence* (Wms & Wilkins, Balt, 52, 252pp, $3). P Nattl Revives *Forgotten Musicians* (Philo Libr, NY, 51, 358pp, $4.75). RL Pullen considers *The Internship* (CC Thomas, Springfield, Ill, 52, 35pp, $1.5). J Strachey translates S Freud's *Totem & Taboo* (Norton, NY, 52, 182 pp, $3).

CLINICAL: CA Bosselaar & N Spronk describe physical method for estimating motility & concentration of spermatozoa (*Nature* 169/18/Jan 5/52).

LJ Cass & LP Wolf find psyllium seed better than other types of laxatives (*Gastroenterol* 20/149/52). HA Christian (1876–1951 reviews primary pericarditis as swan song (*Am Heart J* 42/645/51). FH Ellis & Co analyze role of bronchial arteries in pulmonary embolism & infarction (*Surg* 31/167/52). GM Findlay & Em Howard find cortisone & ACTH increase polio virus growth & severity of symptoms (*J Pharm & Pharmacol* 4/37/52). Sir Francis Fraser dilates on power & responsibility in medicine (*Lancet* 1/61/Jan 12/52). FW Furth & Co report helpful possibilities of aureomycin in radiation syndrome (*Am J Path* 28/25/52). HL Gibson describes *The Photography of Patients* (CC Thomas, Springfield, Ill, 52, 118pp, $5). RH Girdwohl reviews factors influencing megablastic anemias (*Blood* 7/77/52). WEB Hall notes hazard of cholinesterase inhibiting insecticide diethyl-p-nitrophenyl thionophosphate, parathion (*Ann West Med Surg* 5/1025/51). RHJ Hamlin tells how to prevent eclampsia (*Lancet* 1/64/Jan 12/52). RG Hopkinson notes influence of experience on sensitivity to discomfort (*Nature* 169/40/Jan 5/52). NE McKinnon suggests failure of cancer control in case-finding programs (*Surg Gyn Obs* 94/173/52). K Raby shows fall in serum potassium during antabus-alcohol reaction (*Acta Pharmacol Tox* 8/85/52). L Schalin & Co describe regenerative power of liver & bile excretion reserve (*Lancet* 1/75/Jan 12/52). IL Sheckmeister & Co find increased susceptibility to air-borne infections after total radiation (*J Immunol* 68/87/52). JN Swanson & Co evaluate eosinophil counts (*Lancet* 2/129/Jan 19/52). Note Symposium on folic acid antagonists in treatment of Leukemia (*Blood* 7/97–190/52). GW Thorn reviews adrenals in health & disease (*Trans Stud Coll Phys Phila* 19/95/52). CEA Winslow discusses *The Cost of Sickness & the Price of Health* (WHO monograph series No. 7, Geneva, 51, 106pp, $1.5). World Health Organization issues *International Pharmacopoeia* (Vol I, 424pp, Geneva, 52, $5).

APRIL, 1952

GENERAL: R Adams senses man's synthetic future (*Science* 115/157/Feb 15/52). Educational course on medicine in industry uses snappy reportorial style (*Med Bull Esso* 12/1–205/52). WJ Humphreys discusses *Fogs, Clouds & Aviation* (Wms & Wilkins, Balt, 52, 212pp, $3). M Moore acknowledges *Illegitimate Sonnets* (Twayne Publ, NY, 52, 106pp, $2.75). National conference on aging issues *Man & His Years* (Health Publ Inst, Raleigh, NC, 200pp, $1.75). Professional training for general medical practice gets boost (*South Med J* 45/250/52). R Redfield reveals implications of primitive world view (*Proc Am Philosoph Soc* 96/30/52). H Weyl discusses *Symmetry*, basic factor in nature & art (Princeton Univ Press, 52, 175pp, $3.5).

CLINICAL: HG Armstrong revises *Principles & Practice of Aviation Medicine* (Wms & Wilkins, Balt, 52, 488pp, $7.5). KA Baer offers annotated bibliography on *Plasma Substitutes* (Army Med Libr, Washington, 51, 141pp). FM Burnet suggests polio prophylaxis by living attenuated virus

given orally in infancy (*Med J Austral* 1/169/Feb 9/52). CR Denton & Co describe inhibition of melanin formation by hydroquinone benzyl ether (*J Invest Dermatol* 18/119/42). L Dods discusses prenatal pediatrics (*Med J Austral* 39/205/Feb 16/52). JWL Doust & RA Schneider report studies on physiology of awareness relating to anoxia & sleep levels (*Brit Med J* 1/449/Mch 1/52). LG Goodwin reviews recent progress in chemotherapy of tropical diseases (*J Pharm Pharmacol* 4/153/52). OE Hepler revises *Manual of Clinical Laboratory Methods* (CC Thomas, Springfield, Ill, 52, 395pp, $8.5). RL Levy calls halt on needless restrictions on cardiac patients (*Circul* 5/454/52). J Lowe notes favorable results in leprosy from tibione (Aceto-amido-benzyldehyde-thiosemi-carbazone; how about p-amino-hydrazides?) (*Lancet* 1/436/Mch 1/52). IH Martin discusses cortical neurology in regard to visual disturbances (*Med J Austral* 139/211/Feb 16/52). CA Moyer summarizes *Fluid Balance: A Clinical Manual* (Yearbook, Chicago, 52, 191pp, $2.5). JM Robson & AA Sharf note ACTH & cortisone can interrupt pregnancy (*J Physiol* 116/236/52). Note Symposium on cerebral palsy (*J Pediat* 40/340–375/52). WM Wallace reviews intracellular fluid (*Pediat* 9/141/52). EL Way & WC Herbert show pentobarbital reduces antihistamine toxicity (*J Pharmacol* 104/115/52). CJ Wiggers reviews dynamics of ventricular contraction in abnormal conditions (*Circul* 5/321/52).

Preclinical: N Arley & S Iverson develop hit theory of carcinogenesis (*Acta Path Microb Scand* 30/21/52). MAB Brazier discusses *The Electrical Activity of the Nervous System* (Macmillan, NY, 52, 230pp, $5). AM Clark wisely reviews studies on a chemical continuum, referring to redox equilibria (*Bull Johns Hopkins Hosp.* 90/228/52). JH Copenhaven & HA Lardy study high energy phosphate generation in oxidation of metabolites by liver mitachondria (*J Biol Chem* 195/225/52). RH DeMeio & L Tkacz show phenol conjugates more as sulfate than as glucuronate in liver (*ibid* p175). R Fischer & P Larose study cytoplasmic membrane of bacteria & define bactericidal action (*Canad J Med Sci* 30/86/52). J Gross & Pitt-Rivers identify triiodothyronine as usual plasma constituent (*Lancet* 1/439/Mch 1/52). ES Guzman Barron edits *Modern Trends in Physiology & Biochemistry*, in memory of L. Michaelis 1875–1950 (Academic Press, NY, 52, 560pp, $8.5). EN Harvey offers *Bioluminescence* (Academic Press, NY, 52, 665pp, $13). A Hughes describes *The Mitolic Cycle* (Acad. Press, NY, 52, 240pp, $6). Note *International Review of Cytology*, Vol 1, No 1, Feb. 1952 (Acad. Press, NY, $7 per Vol). Also *Labora-Investigation*, Vol 1, No 1, 1952 (PB Hoeber, NY, $8 per Vol). HR Marston reviews cobalt, copper & molybdenum in nutrition (*Physiol Rev* 32/66/52). EW Maynert & Co study metabolism of barbiturates (*J Biol Chem* 195/389, 403/52). J Mellgren & G Pellegrini show anterior pituitary in stress over-produces ACTH & is affected by adrenal steroids (*Acta Path Microb Scand* 30/68/52). RA Peters gives Croonian Lecture on lethal synthesis involving action of fluoroacetate (*Proc Roy Soc B* 139/143/52). G Reid & M Rand suggest that serum vasoconstrictor (serotonin) is tryptamine (*Austral J Exp Biol*

Med Sci 29/401/51). HG Thornton heads talks on micro-organismal symbiosis (*Proc Roy Soc B* 139/170/52). A Trabucco & F Marquez show glomerular tuft in kidney formed by single vessel with evaginations joining common sinus (*J Urol* 67/235/52).

MAY, 1952

SPRING MEETINGS: The American Association of Anatomists offers rich abstracts of reports at annual meeting (*Anat Rec* 112/303–475/52). Ditto for American Association for Cancer Research (*Cancer Res* 12/243–313/52). Federation of American Societies for Experimental Biology overwhelms with 514 page collection of abstracts for annual meeting (*Fed Proc* 11/3–487, plus 25 pages index/52).

BASIC STUFF: S Dack & DH Paley review electrokymography (*Am J Med* 12/331/52). VA Drill discusses mechanisms of action & dietary interrelationship of hepatotoxic agents (*Pharmacol Rev* 4/1/52). ED DeLamater describes mitosis in bacteria (*J Invest Dermatol* 18/225/52). AM ElMofty obtains crystalline aromatic compound (ammoidin) from *ammi majus* with specific effectiveness (caution on sun exposure) in leucoderma (*J Roy Egypt Med Asso* 35/1/52). Our ET Everett & Co report effects of allergens on skin in tissue culture (*J Invest Dermatol* 18/193/52). JW Harmon & M Feigelson continue studies on structure & function of mitachondria (*Exp Cell Res* 3/47/52). MH & FN Jones fail to confirm F Allen & M Weinberg (*Quart J Exp Physiol* 15/385/25) on evidence for four taste systems & their relative sensitivity (*Science* 115/355/Mch 28/52). D Lester & Co evaluate antidotes for alcohol reaction syndrome in patients treated with disulfiram (tetra-ethyl-thiuram disulfide) (*Quart J Stud Alc* 13/1/52). W Lester Jr & Co find propylene glycol vapors is more rapidly acting germicide than related glycols (*Science* 115/379/Apr 4/52). EK Marshall Jr discusses dosage schedule of chemotherapeutic agents, noting no satisfactory data on antibiotics (*Pharmacol Rev* 4/85/52). M Moskowitz & M Cabin separate 3 fractions from red cell membranes (*Exp Cell Res* 3/33/52). O Lindberg & L Ernster describe mechanism of phosphorylative energy transfer in mitachondria (*Exp Cell Res* 3/209/52). MH Nathanson & H Miller note pressor & vagal action of methoxamine (*Am J Med Sci* 223/270/52). Our CM Pomerat demonstrates rhythmic pulsating cells in human brain cortex tissue culture (*Anat Rec* 112/473/52).

OF GENERAL INTEREST: AL Blakeslee gives pertinent material in *Alcoholism: A Sickness That Can Be Beaten* (*Public Affairs Com.* NY, 52, 32pp, 25c). EA Boyden writes worthy appreciation of Frederic T Lewis 1875–1951 (*Anat Rec* 112/137/52). SL Deignan & E Miller analyse support of research in medical fields in USA fr 1946 to 1951 (*Science* 115/321/Mch 28/52). C Elgood offers *A Medical History of Persia & the Eastern Caliphate* (Cambridge Univ Press, 51, 628pp, $10). GJ Heuer (1882–1951) reminisces on WS Halsted (1852–1922) (*Bull Johns Hopkins Hosp*,

Suppl 90/1–105/Feb 52). D. Nachmansohn & Co eulogize Otto Meyerhof (1884–1951 (*Science* 115/365/Apr 4/52). O Neugebauer describes *The Exact Sciences in Antiquity* (Princeton Univ Press, 52, 236pp, $5). R Niebuhr well exposes *The Irony of American History* (Scribner's, NY, 52, 174pp, $2.75). J Parr tells *How I Cured My Ulcer* (Little Brown, Boston, 52, 155pp, $2.75).

Clinical Information: J Bernstein, W Steenken, WM Benson, B Rubin, EH Robitzek, DF Elmendorf & large Co report on value in tuberculosis of isonicotinic hydrazides (*Am Rev Tuberc* 65/357–442/52). WL Hubler & Co show fever stimulates pituitary & adrenal cortex (*Blood* 7/326/52). DD Jackson & Co indicate post-irradiation clotting defects are due to platelet loss (*J Lab Clin Med* 39/449/52). A Kattus & Co note favorable oral diuresis from propyl-ethyl-amino-uracil (*Am J Med* 12/319/52). RL Kahn, FJ Hodges & Co show that patients whose universal serologic reactions fall following radiation exposure have poor prognosis (*Cancer Res* 12/170/52). JN Morris & Co ably discuss coronary heart disease in medical men (*Brit Med J* 1/503/Mch 8/52). I Starr & EA Hildreth find that heart on aging lifts its load ever more slowly (*Circula* 5/481/52).